THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 124 OCTOBER 1, 1938 No. 1

# QUANTITATIVE STUDIES OF EXPERIMENTAL MENSTRUATION-LIKE BLEEDING DUE TO HORMONE DEPRIVATION[1]

GEORGE W. CORNER

*From the Department of Anatomy, The University of Rochester, School of Medicine and Dentistry*

Received for publication June 6, 1938

The following experiments were undertaken to test a hypothetical explanation, or partial explanation, of the rôle of the ovarian hormones in the menstrual cycle. We know from the experiments of E. Allen (1926) and many others (see Corner, 1933, for review of the evidence) that menstruation-like bleeding can be produced in monkeys by various procedures involving a sudden deprivation of estrin; for example, by bilateral oöphorectomy, or in a castrate monkey, by discontinuing a course of estrin treatment. This observation suggested the estrin-deprivation theory of menstruation in its original form, which postulates simply that menstrual bleeding is due to a periodic lowering of the estrin level of the body. On this hypothesis it should be possible to inhibit normal menstruation by administration of estrin. Attempts of the present author (Corner, 1935) to produce such inhibition yielded negative results; that is to say, daily doses of estrin, presumably within the physiological range, did not interfere with the first menstrual period due after the beginning of the injections. (For a criticism of this interpretation, see Zuckerman, 1936; for data supporting the validity of the dosage used, see Allen, Diddle, Burford and Gardner, 1936.)

On the other hand, administration of progesterone in small dosage promptly inhibits natural menstruation in experimental monkeys (Corner, 1935; Corner and W. M. Allen, 1936). Meanwhile it had been shown (Smith and Engle, 1932; Engle, Smith, and Shelesnyak, 1935) that in castrate monkeys bleeding following estrin deprivation can be prevented

[1] This work was made possible by a grant in support of the author's work made to the University of Rochester by the Rockefeller Foundation, Division of Natural Sciences.

by treatment with the hormone of the corpus luteum. These authors used crude extracts; the observation was confirmed with practically pure progesterone by Hisaw (1935). Its confirmation with crystalline progesterone is reported in the present paper.

The discontinuance of an effective course of progestin injections invariably causes menstruation-like bleeding (Smith and Engle, 1932; Engle, Smith and Shelesnyak, 1935; Corner, 1935; Zuckerman, 1937a, b, c).

In summary, estrin deprivation in the castrate monkey produces menstruation-like bleeding, and so does progesterone deprivation. Natural menstruation is however not readily inhibited by estrin, but is inhibited by progesterone.

These experimental findings obviously do not support the estrin-deprivation hypothesis of menstruation in the simple form cited above, but as the reader will perceive, they can be made to furnish an almost equally simple explanation of the normal menstrual cycle. We have learned that both estrin and progestin possess the power, by reason of their respective effects upon the endometrium, to prevent the deprivational breakdown.

Let us now assume that progestin in some way or other has the property of suppressing the menstruation-preventing power of estrin, while itself holding off menstruation. Then in the normal cycle the uterus will not bleed during the first half ("follicular phase") because the ovaries are furnishing estrin. It will not bleed during the second half of the cycle ("corpus luteum phase") because the corpus luteum is furnishing progestin. The production of estrin in all probability continues. By our assumption, however, the corpus luteum is suppressing the protecting effect of estrin; therefore when the corpus luteum undergoes retrogression, the animal is suddenly deprived of the action of both estrin and progesterone, and the endometrium breaks down.

This modified deprivation hypothesis of menstruation was presented to the National Academy of Sciences at Washington in April, 1937 (Corner, 1937) with evidence comprising part of the experiments to be described herewith. The crux of the hypothesis is the assumption that progestin acts to prevent estrin from protecting the endometrium against bleeding. It is not to be expected that such a reaction would be absolute, i.e., independent of the quantities of the two hormones acting at a given time; on the contrary it would, in all probability, operate only when they were present in a specific and critical ratio. The hypothesis has had therefore to be tested by tedious and expensive quantitative experiments requiring more than two years. Other workers, notably Zuckerman, Hisaw, P. E. Smith and Engle, have been following somewhat similar lines of thought, as indicated by their papers to be cited herewith and by remarks at scientific meetings. Zuckerman (1937a, c) has stated clearly a conception of the inter-reaction of estrin and progesterone practically identical with

that outlined by the present author in the same year. The present report makes therefore no claim to novelty, either of ideas or of experimental results, but will be of value (it is hoped) because it is based on a systematic program of quantitative experiments aimed directly at the special problem in hand.

EXPERIMENTS. The animals used were 11 female rhesus monkeys, all of which had passed through one or more menstrual cycles before they were castrated for use in the experiments. The estrogenic hormone was estrone, the preparation used being Amniotin (Squibb) in oil. The progesterone used in the experiments came chiefly from a generous supply of the synthetic hormone, put at my disposal by the Schering Corporation through Dr. Erwin Schwenk. I take this occasion to express thanks for the gift. Merck and Company also very kindly provided a supply of the hormone. A few experiments were done with natural crystalline progesterone prepared in our laboratory by Dr. Willard M. Allen. We have had sufficient experience with the effects of these preparations to be certain that they are chemically and physiologically identical. The progesterone was dissolved in sesame oil as required for injection. To save repetition later it may be stated here that the sesame oil can be excluded as a possible cause of the inhibitory effects under consideration, in view of numerous negative experiments when the amount of hormone in the oily injection was reduced below the effective limit. As an extra precaution, tests with sesame oil alone were made.

*Standard estrin-deprivation experiment.* The first step was to adopt a standard procedure by which estrin-deprivation bleeding could be produced, with perfect reliability but with the use of as small a quantity of estrogenic substance as possible. The dosage adopted for the present experiments was 125 international units of estrone in the form of Amniotin in oil, daily for 10 days, making a total of 1250 international units. The report of E. Allen, Diddle, Burford, and Gardner (1936) states that in oöphorectomized mature monkeys doses of 81 to 122 rat units of estrin in oil, given over 9 to 14 days, proved adequate to induce estrin-deprivation bleeding. Although the factor for converting rat units into international equivalents is variously estimated from 3 to 10 by different observers, it will be seen that our standard procedure employs a dosage somewhat larger, no matter what the basis of comparison, than the minimum adequate dosage worked out by E. Allen and his colleagues.

Every animal used in the experiments, with one exception, was subjected to the standard estrin-deprivation experiment at least once, and some of them more often. In all there were 20 trials on the 10 animals. In every case vaginal bleeding occurred after discontinuance of the injections, the latent period varying from 5 to 8 days if microscopic bleeding is accepted as the criterion, and from 5 to 10 days if macroscopic bleeding is the test.

One animal which was tried 5 times bled macroscopically on the 6th day after discontinuance once, on the 7th day twice, the 8th day once, the 10th day once.

*Effects of progesterone combined with estrone.* The experiments of this group were performed as follows: The basic daily dose of 125 int.u. of estrone was given daily for 10 days, then 1 mgm. of progesterone was given together with the 125 int.u. estrone daily for 10 days. After discontinuance of the progesterone, the estrone was kept up, at first in the same daily dose of 125 int.u. After it was discovered that such a quantity

TABLE 1

*Estrone 125 int. u. for 10 days, then progesterone 1 mgm. in conjunction with estrone 125 int. u. for 10 days, followed by estrone alone in dosages indicated below*

In column 3, figure in parenthesis indicates microscopic bleeding

| ANIMAL | FINAL ESTRONE DAILY | BLEEDING DURING FINAL ESTRONE |
|---|---|---|
| | *int.u.* | |
| 148 | 250 | (3d) 4th day |
| 155 | 250 | 4th day |
| 155 | 250 | 4th day |
| 155 | 400 | 3d day, slight |
| 153 | 500 | None |
| 137 | 500 | 8th day, slight |
| 148 | 500 | 4th day, slight |
| 153 | 575 | None |
| 148 | 575 | 4th |
| 148 | 600 | (4th) 5th day |
| 155 | 600 | 3d day, microscopic |
| 153 | 650 | None |
| 148 | 650 | 4th day |
| 127 | 650 | None |
| 153 | 750 | None |
| 148 | 750 | (5th) 6th day, slight |

In each of the 5 cases in which no bleeding at all occurred during the final course of estrone, bleeding took place 6 to 9 days after withdrawal of estrone, showing that the animal was susceptible of ordinary estrone-deprivation bleeding.

would now not prevent bleeding, the dose during this third phase of the experiment was raised as indicated by the appropriate column in table 1.

In addition to the tabulated experiments, two controls were run by administering sesame oil instead of progesterone. Two other experiments, in which ineffective amounts of progesterone, in sesame oil, were given, may also be considered as controls. In all 4 experiments no bleeding occurred during the 35 days of estrone injections.

Table 1 reveals the striking fact that *a course of progesterone sets up a condition such that after withdrawal of the corpus luteum hormone bleeding cannot be prevented by doses of estrone normally adequate.* The daily dose

of estrone must be raised to 500 or more international units, and in some animals, e.g., no. 148, as much as 750 units daily will not entirely prevent endometrial bleeding after cessation of the course of progesterone.

This result, which conforms to the hypothesis set forth above, agrees with the experimental findings of Engle, Smith and Shelesnyak (1935), Hisaw (1935), and Zuckerman (1937c). The first-named authors report 6 experiments in which a course of progestin, approximating 1 to 2 international units daily, was discontinued, but immediately followed by estrone (Theelin) 100–200 rat units daily. Bleeding occurred in spite of the estrone treatment, beginning on various days from the 4th to the 11th day after the last injection of progestin. Hisaw cites one similar experiment (his monkey AL.16) and in personal letter to the author has very kindly mentioned a considerable number of others, of which accounts will appear in his papers now in press and in preparation. Zuckerman (1937c) lists in table 1 of his paper 8 relevant experiments, similar in plan to those of the present author, but executed with somewhat different quantities of the hormones and times of administration. The monkeys were given large doses of estrone (1000–3000 int.u.) daily for 14 days, then dropped to smaller doses (50–1000 units) together with 0.5 to 20 mgm. of progestin daily for 10–12 days. After discontinuance of the progestin, the dosage of estrone was maintained or raised, and it was found that in 7 of the 8 trials bleeding set in in spite of as much as 500 int.u. of estrone daily. In one case even 1000 int.u. were powerless to inhibit the bleeding, but the monkey had received the enormous quantity of 20 mgm. of crystalline progesterone daily, with 1000 int.u. of estrone, for 12 days.

This observed fact, that progesterone-deprivation bleeding cannot be inhibited by estrone in amounts sufficient to prevent estrin-deprivation bleeding, might be explained in two ways. One explanation is that of our original hypothesis, namely, that progesterone exerts some sort of action which suppresses the protective effect of the estrogenic substance. The other possible explanation is that when we administer estrin and progesterone together we are simply administering an increased dosage of protective substances. In the foregoing experiments, for example, 125 international units of estrone plus 1 mgm. of progesterone may, for all we know, afford protective power against deprivation bleeding equivalent to a dose of estrone alone far larger than 125 units; when discontinued one might naturally expect that a large daily dose of estrin would be required. This conjecture assumes that the protective powers of the two hormones are of the same order and can be summated arithmetically. There may be a fallacy here like the school-child's difficulty about adding horses and cows; and there is, moreover, another uncertain factor in the situation, to be discussed below. The following experiments were however undertaken to analyze the problem.

*Amount of progesterone required to prevent estrin-deprivation bleeding.* Eighteen experiments were done on six animals. In each experiment the animal was given the standard ten day course of estrin, after which, beginning on the day after the last dose of estrin, progesterone was administered in equal daily doses for 15 days or until bleeding occurred.

Since in our standard estrin-deprivation experiment bleeding always occurred on or before the 10th day of deprivation, in the present and following groups of experiments any postponement of bleeding beyond the 10th day after the last 125 unit dose of estrone may be considered to indicate an effect of the hormone subsequently injected. For the sake of certainty,

TABLE 2

*Inhibition of estrin deprivation bleeding by progesterone*

In last column, figure in parenthesis indicates microscopic bleeding

| ANIMAL | DAILY DOSE | BLEEDING DURING ADMINISTRATION PROGESTERONE | BLEEDING AFTER DISCONTINUANCE PROGESTERONE |
|---|---|---|---|
| | *mgm.* | | |
| 127 | 1.0 | Inhibited | 4th day |
| 136 | 1.0 | Inhibited (injected 13 days only) | (2d) 3d day |
| 137 | 0.5 | Inhibited | 5th day |
| 132 | 0.5 | Inhibited | (6th) 10th day |
| 137 | 0.5 | Inhibited (a few r.b.c. on 9th-11th day) | (1st) 2d day |
| 132 | 0.35 | Inhibited | (3d) 5th day |
| 137 | 0.35 | Began 13th day | |
| 147 | 0.35 | Inhibited | (13th) 14th day |
| 147 | 0.35 | Inhibited | (5th) 6th day |
| 132 | 0.25 | Inhibited (injected 10 days only) | (3d) 5th day |
| 127 | 0.25 | Inhibited (injected 10 days only) | 3d day |
| 147 | 0.25 | Trace bleeding 12th, 13th day | |
| 176 | 0.25 | Began 9th day | |
| 127 | 0.25 | Inhibited | 1st day |
| 132 | 0.25 | Began 11th day | |
| 132 | 0.125 | Began 8th day | |
| 137 | 0.125 | Began 9th day | |
| 127 | 0.125 | Began (10th) 11th day | |

however, the second phase of the experiment was continued for 15 days and bleeding has been considered to be definitely inhibited only when there was no bleeding during the whole of that period. Three experiments which ran less than 15 days are noted in table 2.

It will be seen that progesterone regularly inhibited the bleeding of the standard estrin-deprivation experiment, when given in daily doses of 0.5 mgm. or more. Thirty-five hundredths milligram inhibited in 2 of 4 experiments; 0.25 mgm. failed to inhibit in 3 of 4 cases and in the fourth case barely carried the animal through the 15 day period; 0.125 failed to inhibit in 3 cases.

Under the conditions of the experiment, the critical dosage, i.e., the dosage at which there is an even chance that inhibition will result, is 0.35 mgm. This figure is derived from the total aspect of the group; for any given animal it appears that the critical dose may vary somewhat from the average. Critical dosage for 4 of the monkeys, drawn from the above list, will be seen in the first column of table 3.

*Amount of estrin required to prevent estrin-deprivation bleeding.* In this group of experiments each animal was subjected to the standard 10 day course of estrin. On the 11th day the dosage of estrin was changed to

TABLE 3

*Results of diminution of estrin dosage*

| ANIMAL | CRITICAL DOSE PROGESTERONE (FROM TABLE) | ESTRONE | | RESULTING BLEEDING |
|---|---|---|---|---|
| | | Priming dose | Reduced dose | |
| | *mgm.* | *int.u.* | *int.u.* | |
| 127 | 0.25 | 125 | 62.5 | Inhibited |
| | | 125 | 50 | Inhibited |
| | | 125 | 25 | Trace after 9th day |
| 132 | 0.25 | 125 | 75 | 13th day |
| | | 125 | 50 | Inhibited |
| | | 125 | 50 | (9th) 10th day |
| | | 125 | 37.5 | (9th) 13th day |
| | | 125 | 25 | 8th day |
| 137 | 0.5 | 125 | 100 | Inhibited |
| | | 125 | 75 | Inhibited |
| | | 125 | 75 | Inhibited |
| | | 125 | 62.5 | (14th) 15th day |
| | | 125 | 50 | 9th |
| 147 | 0.35 | 125 | 100 | (13th) 14th day |
| | | 125 | 75 | 11th day |
| | | 125 | 62.5 | 10th day |
| | | 125 | 50 | 5th day |

a smaller amount and continued for 15 days. By thus determining the critical dosage of estrin required to prevent estrin-deprivation bleeding it becomes possible to compare the protective potency of estrin with that of progesterone as determined in the previous experiments.

A few preliminary tests revealed that the ability of the animals to withstand a drop in estrin level without bleeding differs greatly in individual monkeys. For this reason the table is based on four animals which were subjected to repeated trials in order to get an idea of the individual sensitivity. The response of these animals to varying doses of

progesterone after estrone deprivation had already been determined; it is shown in table 2 (nos. 127, 132, 137, 147) and is summarized in table 3, column 2. A few experiments on other individuals, though not listed, are in complete accord with these.

These results are not very consistent. Obviously the basic dose of 125 int.u. of estrone daily is not far above the threshold dose for the prevention of bleeding, and when the dosage is reduced, from 50 to 100 int.u. may be required to prevent estrin-deprivation bleeding. Comparing the protective effects of progesterone and of estrone in these experiments, it appears that 0.25 mgm. of progesterone equals about 50 int.u. of estrone. At the most (as in the case of animal 147, table 3) 0.35 mgm. progesterone equals about 100 int.u.

The foregoing result with regard to the quantitative aspect of a reduction in estrin dosage is in agreement with a series reported by Zuckerman (1937b).

The figures obtained may now be applied to the problem of the possible additive effects of estrin and progesterone in the prevention of deprivation bleeding. From the experiments listed in table 3, in which 0.35 mgm. to 0.25 mgm. of progesterone was found to give the same effect as 100 international units of estrone, then (granting the propriety of dealing with these effects by simple arithmetical operations) 1 mgm. of progesterone is physiologically equivalent, in prevention of uterine bleeding, to 200 to 300 int.u. of estrone. Therefore when we gave 125 int.u. of estrone plus 1 mgm. of progesterone we were giving a total protective power equivalent to 325 or at most 425 international units of estrone. It was found however that discontinuance of the combined hormones could not be compensated for by less than 500 or in some cases even 750 international units of estrone. The administration of progesterone therefore has caused an effect greater than can possibly be explained on the basis of its own protective power against deprivation bleeding.

As hinted above, there was another uncertain factor to be considered. The explanation on the basis of addition of protective powers assumes that deprivation bleeding will be caused by a relative lowering of hormone level, i.e., the drop from say 325 equivalent units (as the summated potencies turned out to be) to 125 units of estrone is expected to produce the same effect as the drop from 125 units to 50 units of estrone. Whether this is true was however quite unknown when the foregoing experiments were under way. The author therefore began studying the effect of lowering the estrone level from relatively high doses to lower doses, thus causing estrone reduction of greater absolute quantity than in the experiments of table 3 but of similar proportions. Extensive work was however rendered unnecessary by the appearance of Zuckerman's studies of the estrone threshold of the uterus of the rhesus monkey (1937c). From his tables

it appears clearly that estrin-deprivation bleeding is not dependent upon proportionate reduction of estrin level; on the contrary no matter how high the primary dosage, bleeding will result only when the reduced level is below a certain threshold value which under his experimental conditions was about 100–150 int.u. daily. For example, a drop from 3000 to 200 int.u. did not usually cause bleeding, while a drop from 3000 or even 500 to 50 or to 100 units did cause bleeding. A few experiments by the present writer gave entirely similar results.

In our estrone-progesterone-estrone sequence, the final daily dosage of estrone necessary to prevent bleeding was far above the threshold level, again suggesting that the progesterone-deprivation bleeding is not to be explained as due simply to lowering of the total hormone level by removal of an activity akin to that of estrone and measurable in the same quantities.

Discussion. It is concluded from the foregoing experiments that when estrone and progesterone are administered at the same time, in suitable dosage, to castrated female monkeys, the progesterone in some way suppresses the effect of the estrone on the endometrium, while itself preventing estrin-deprivation bleeding.

The idea of opposing or antagonistic action of the two ovarian hormones is not new, and rests upon a considerable mass of evidence. M. G. Smith (1926), Parkes and Bellerby (1926), and Courrier (1930) called attention to the fact that estrin administered during the first few days of pregnancy or pseudo-pregnancy will prevent the histological effects of the corpus luteum, and will terminate pregnancy. Hisaw and Leonard (1930), Tausk, de Fremery and Luchs (1931), and Leonard, Hisaw and Fevold (1932) carried the observation further by demonstrating that the progestational effect of progestin on the castrate rabbit's uterus can be prevented by estrin. Robson (1936), working with pure hormones, has worked out the precise quantitative relationship involved in the reaction. Studies of Courrier and his colleagues begun in 1930 (summarized by Courrier, 1935) on the formation of experimental deciduomata in castrate animals under the influence of progestin, showed that this effect of the corpus luteum hormone can be antagonized by estrin. Courrier also reviews various experiments which point to an antagonism in the opposite direction: in rats, mice and guinea-pigs a dose of estrogenic hormone adequate to cause the typical vaginal reaction will prove inadequate if given when the animal's ovaries contain functional corpora lutea. W. M. Allen and Meyer (1935) carried out a similar experiment even more convincingly by using castrate mice and supplying the progestin by injection. It has become well known also that the two hormones have diametrically opposite effects upon the spontaneous motility of the uterine musculature, estrogenic substances enhancing muscular contractions, progesterone inhibiting them (for review, see Reynolds, 1937).

Exactly how such counteractive effects may be produced is a matter for separate consideration. It may be very misleading to let the matter rest upon the concept of a specific antagonism. Each case will require investigation separately and it may even turn out that the mechanism by which one hormone limits the action of the other is not always the same. Direct chemical antagonism, such as neutralization of one hormone by the other, is not likely, in view of their chemical similarity. Smith and Smith (1931) suggested that progestin lowers the estrin level by making the kidney more permeable to estrin, but so far as known to the present writer, the experiments still await confirmation. Leonard, Hisaw and Fevold (1932) speak of the two hormones as "competing for the cells of the endometrium." The blockade may conceivably occur in the receptor tissues, i.e., one of the hormones may create such a special state in (for example) the endometrium, that the other can no longer affect it. To speculate further, such a change may be at a physico-chemical level, depending on molecular states in the tissues, or it may be at a morphological level; that is to say, using our present case as an example, the histological effects of progesterone may be so different from those of estrone that their retrogression cannot be averted by the latter hormone.

If the last-mentioned situation occurs in our experiments, then the bleeding which follows discontinuance of progesterone is truly progesterone-deprivation bleeding; but if the former situation occurs (i.e., progesterone drives out estrone or blocks its action by some sort of direct antagonism) then the bleeding which follows discontinuance of progesterone is simply a special case of estrin-deprivation bleeding.

Which explanation is correct cannot now be stated. In the present experiments biopsy of the uterus was not done, because the resultant surgical hemorrhage would have confused the results; it is not known therefore whether at the end of the course of progesterone combined with estrone there were morphological changes (premenstrual state) in the endometrium. Zuckerman (1937c) examined the endometrium in a few of his cases, and found that the histological effects of the estrone-progesterone combinations used were inconsistent, a fact which he explains by the uncertainties due to mutually antagonistic action of the two hormones. Professor Hisaw in personal letters to the author states that his experiments indicate that the progesterone effects which make it difficult to inhibit bleeding by injecting estrin following progesterone withdrawal, occur independently of glandular changes. Engle (1938) states that in the experiments of Engle, Smith, and Shelesnyak (1935) cited above, an "acceptable endometrium" (i.e., premenstrual state) had been produced by the combined estrin and progesterone. It is well known to workers in this field, however, that it is not easy to hit upon a correct combination to produce the premenstrual state, whereas the proportionate dosage

required to produce estrin-resistant deprivation bleeding is evidently not very critical. For this reason it seems, at present writing, likely that under the conditions of the present experiment (and in normal ovulatory menstrual cycles as well) the occurrence of the premenstrual state is not the critical factor and that the significant action of the progestin has been to create, by depressing the action of estrin, a state equivalent to estrin deprivation. Further experiments aimed at exploring this question are under way.

## SUMMARY

When estrone and progesterone are administered at the same time, in suitable dosage, to castrated monkeys, discontinuance of the progesterone is followed by menstruation-like uterine bleeding in spite of the continuance of estrone treatment at a level more than sufficient to prevent estrin-deprivation bleeding. This result cannot be explained by assuming that discontinuance of progesterone has merely amounted to lowering of the total hormone level. It must be assumed that progesterone in some way suppresses the action of the estrogenic hormone upon the endometrium, while itself preventing uterine breakdown, so that when the progesterone is discontinued, the animal is then deprived of the action of both hormones. It remains to be determined whether the bleeding which follows a course of progesterone is a specific progesterone-deprivation effect or merely a special case of estrin-deprivation bleeding.

Normal menstrual bleeding of ovulatory cycles may be explained, on the basis of this interpretation, as due to the periodic action and retrogression of the corpus luteum, without necessarily postulating alternations in the rate of production of estrogenic hormone by the ovary. Proof of such an explanation must necessarily await determination of the amounts of estrin and progestin actually present at the various stages of the primate cycle.

*Author's note:* Since the preparation of the foregoing paper Hisaw and Greep (Endocrinology **23:** 1, 1938) have published in detail the work referred to above on the basis of personal communications. In view of their demonstration that the effects of progesterone which make it difficult to inhibit bleeding by subsequent injection of estrin occur independently of, and earlier than the glandular changes, they suggest that the effects in question may be due to a modification of the endometrial stroma by progesterone.

## REFERENCES

ALLEN, E. Proc. Soc. Exper. Biol. Med. **23:** 434, 1926.

ALLEN, E., A. W. DIDDLE, T. H. BURFORD AND W. U. GARDNER. This Journal **117:** 381, 1936.

ALLEN, W. M. AND R. K. MEYER. Anat. Rec. **61:** 427, 1935.

CORNER, G. W. Medicine **12:** 61, 1933. Also in Harvey Society Lectures, 1932–1933, 67.

CORNER, G. W. This Journal **113:** 238, 1935.
Science **85:** 437, 1937.
CORNER, G. W. AND W. M. ALLEN. Proc. Soc. Exper. Biol. Med. **34:** 723, 1936.
COURRIER, R. C. R. Soc. de Biol. **104:** 1178, 1930.
Bull. d'Histol. **12:** 261, 1935.
ENGLE, E. T. Cold Spring Harbor Symposia on Quantitative Biology **5:** 111, 1938.
ENGLE, E. T., P. E. SMITH AND M. C. SHELESNYAK. Am. J. Obst. and Gynec. **29:** 787, 1935.
HISAW, F. L. Am. J. Obst. and Gynec. **29:** 638, 1935.
HISAW, F. L. AND S. L. LEONARD. This Journal **92:** 574, 1930.
LEONARD, S. L., F. L. HISAW AND H. L. FEVOLD. This Journal **100:** 111, 1932.
PARKES, A. S. AND C. W. BELLERBY. J. Physiol. **62:** 145, 1926.
REYNOLDS, S. R. M. Physiol. Rev. **17:** 304, 1937.
ROBSON, J. M. J. Physiol. **88:** 100, 1936.
SMITH, G. V. AND O. W. SMITH. This Journal **98:** 578, 1931.
SMITH, M. G. Johns Hopkins Hosp. Bull. **39:** 203, 1926.
SMITH, P. E. AND E. T. ENGLE. Proc. Soc. Exper. Biol. Med. **29:** 1225, 1932.
TAUSK, M., P. DE FREMERY AND A. LUCHS. Acta Brevia Neerlandica **1,** 1931.
ZUCKERMAN, S. Lancet p. 9, 1936.
ZUCKERMAN, S. J. Physiol. **89,** 1937 a.
ZUCKERMAN, S. Proc. Roy. Soc. London, B, **123:** 441, 1937 b.
ZUCKERMAN, S. Proc. Roy. Soc. London, B, **124:** 150, 1937 c.

# THE OXYGEN SATURATION OF THE VENOUS BLOOD IN NORMAL HUMAN SUBJECTS

ANCEL KEYS

*From The Laboratory of Physiology and Physical Education, The University of Minnesota, Minneapolis*

Received for publication June 7, 1938

The most obvious criterion for evaluating the adequacy or deficiency of the circulation is the arterial-venous difference in the oxygen content of the blood. Ordinarily, the arterial saturation is always between 93 and 98 per cent and 93 per cent of all determinations on normal human subjects at ordinary altitudes are between 94 and 96.5 per cent saturation (cf. Keys and Snell, 1938). The conditions in which there is inadequate arterial oxygenation are relatively few and not difficult to recognize, so an estimate of the venous saturation would seem to be all that is needed to gauge the adequacy of the circulation. Clinical conclusions are not infrequently made on this assumption. This requires, however, a knowledge of the values for normals and implies that the frequency range of normals is relatively constant and restricted.

In human subjects venous blood samples are drawn from the arm in the great majority of cases. Jugular, femoral or saphenous vein punctures, though safe enough in the hands of skilled technicians, must necessarily be limited to special conditions; right heart punctures are impractical for obvious reasons. The present paper attempts to arrive at relatively final values for the frequency range for the oxygen saturation of arm vein blood in normal human subjects in basal rest and the influence of some deviations from basal conditions. Comparisons are also made with blood from the large leg veins.

MATERIAL. The subjects were men and women between the ages of 18 and 50, all of whom knew the nature of the study and were as nearly as possible unconcerned about the manipulations. All were leading normal active lives as students, technicians, clerks, attendants and physicians. Every subject had been given a thorough medical examination within 6 months of the time of the study and had been pronounced free from any signs of a pathological nature. In most of the cases direct measurements of basal metabolism had been made. The past histories of these subjects showed nothing significant except for two cases of long and completely arrested pulmonary tuberculosis. All subjects abstained from alcohol for 24 hours and had a normal night's sleep before the test. In no case had

there been any prolonged exposure to unusual heat or cold for at least several days before the test. All women were studied in the intermenstrual period.

PROCEDURE AND CONDITIONS. The subjects came to the laboratory in the morning directly from their homes. In no case was there any more exertion than that corresponding to a walk, at a moderate pace, for several hundred yards. All subjects were in the fasting state. On arrival at the laboratory the subjects were put to bed in a quiet room and allowed to rest 30 minutes before blood samples were taken. The sampling room was well-ventilated and maintained at a temperature between 23 and 25°C., the room temperature to which all the subjects were habituated.

During the resting period the pulse rate was counted several times and the blood pressure was measured 10 minutes before the venipuncture was made. For 5 to 10 minutes before venipuncture the hand and arm, bared to above the antecubital fossa, rested on the coverlet in a relaxed position. Blood samples were quickly drawn without the application of any pressure or stasis whatever.

With 20 subjects, after the first blood sample was taken, the subject got out of bed and ate a small standard breakfast consisting of 4 ounces grapefruit juice, a large sweet roll with butter, and a cup of coffee with cream and sugar. After breakfast these subjects went to the toilet, wandered about the laboratories for a short time and returned to bed within 25 minutes of arising. Again they rested quietly for 30 minutes before the second blood sample was taken.

With 12 male subjects, after the first blood sample was taken, the circulation was strongly stimulated by brief violent exercise or by the intravenous injection of epinephrine. The subject then returned to bed and a second blood sample was taken after 60 minutes of quiet rest. The exercise was running in place with maximal exertion for 1 minute. The subjects who were given epinephrine received from 0.15 to 0.25 mgm. intravenously, the injection being given slowly over a period of several minutes.

Femoral vein punctures were made on five normal male subjects. The same precautions were observed as in the venipunctures on the arm.

METHOD. Blood was drawn into oiled syringes and immediately heparinized under oil and analyzed. The standard Van Slyke procedure was used for analysis. A part of the blood was saturated for oxygen capacity measurement by slow rotation for 20 minutes with room air in a Barcroft tonometer. Correction was made for oxygen in physical solution both in the venous content and in the oxygen capacity determinations. All analyses were made in duplicate and no results were accepted in which the duplicates differed by more than 0.25 volume per cent of oxygen.

RESULTS. The main series of studies on 45 men and 18 women is

summarized in figure 1. The mean venous saturation in basal rest was 68.2 per cent, range 28.8 to 88.5 per cent. Figure 1 shows that the distribution is markedly skew and though there is only 1 value above 85 per cent, 28.5 per cent of the values are below 60 per cent saturation, in other words, 30 per cent of our subjects were outside the range frequently cited as the "normal limits" (cf. Peters and Van Slyke, 1931, p. 570).

The agreement between duplicate analyses showed that the individual variations were not due to analytical error. The possibility of faults in the technique of sampling or transferring the blood was tested by two means. In four subjects a second venipuncture, on the same arm, was made immediately after the first sample was taken. In five other subjects when the syringe had been filled with blood it was slipped from the needle

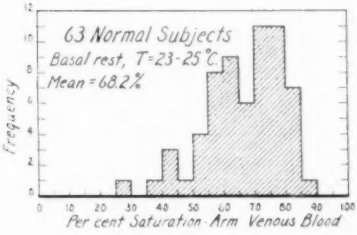



Fig. 1

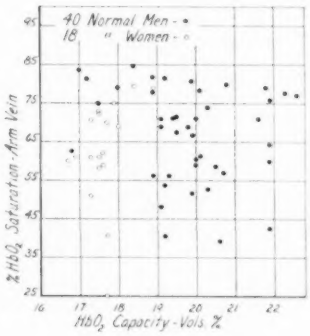



Fig. 2

Fig. 1. Distribution of the oxygen saturations in venous blood from the antecubital fossa in 63 normal adults in the basal state.

Fig. 2. Oxygen saturation of the venous blood from the antecubital fossa versus the oxygen capacity of the blood.

and a second sample taken with a new syringe without making a second venipuncture. In none of these nine cases did the two blood samples differ by as much as 5 per cent in oxygen saturation.

In the present series the number of females (18) may be smaller than desirable, but there seems to be a small but significant difference between the sexes. The r[illegible]tions are shown in table 1. The females tend to have lower venous saturation and, on the average, a slightly higher oxygen utilization per circuit of the blood. For calculation of the latter value I have taken the value of 95 per cent for the arterial saturation in all cases. In view of our finding of the constancy of the arterial saturation, no more than an insignificant error can be introduced by this assumption.

It might be thought that the variations in the venous saturation are related to the level of the total oxygen capacity of the blood. The com-

plete absence of any such relation, both in men and in women, is shown by figure 2.

The oxygen saturation of arm vein blood before and after a light breakfast is not constant, but, in different individuals, there is a high degree of correlation between the two values (fig. 3). The maximum change observed in the present series of 20 subjects was 16 per cent, and the average change was only 5.4 per cent in oxygen saturation.

TABLE 1

*Comparison of venous saturations in normal males and females*

| SEX | NUMBER | VENOUS OXYGEN SATURATION | | | MEAN $O_2$ UPTAKE, CC. PER 100 CC. BLOOD |
|---|---|---|---|---|---|
| | | Mean | Standard deviation | Range | |
| | | *per cent* | *per cent* | *per cent* | |
| F | 18 | 62.8 | ±11.66 | 24.5 to 79.4 | 5.67 |
| M | 45 | 70.3 | ±13.15 | 39.2 to 84.9 | 5.26 |

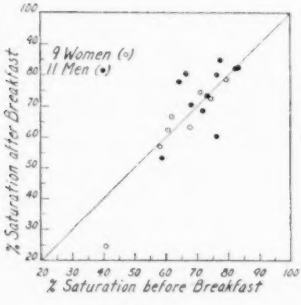



Fig. 3

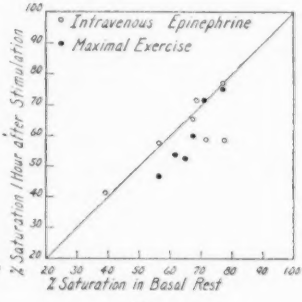



Fig. 4

Fig. 3. Oxygen saturation of the venous blood from the antecubital fossa before and 30 minutes after a light, standard breakfast.

Fig. 4. Oxygen saturation of the venous blood from the antecubital fossa before and 1 hour after brief, extreme stimulation of the circulation by means of exercise and the intravenous injection of epinephrine.

The circulatory disturbance produced by brief maximal exercise or by the intravenous administration of epinephrine is much more profound than results from eating a light breakfast. Nevertheless, after an hour's rest the level of venous saturation returns to a value not far from that preceding the exercise or the epinephrine. As shown in figure 4, however, the second venous saturation is generally lower than before the circulation was stimulated. The mean change one hour after exercise or epinephrine was a fall of 6 per cent in the venous saturation in the arm vein blood.

Variations in the oxygen saturation of venous blood from the leg—

femoral and great saphenous veins—may be gauged from figure 5. These results, all on "normal" males in the resting state are from several sources; Florkin, Edwards and Dill (1930) studied 10 subjects, Theis and Freeland (1937) give data for 6 subjects, and 5 subjects were studied by me. It is obvious that the variations in oxygen saturation of leg vein blood are closely similar to those in the arm vein bloods (see also Weiss and Ellis, 1935).

Figure 5 presents also a summary of the acceptable data for the resting oxygen saturation in arm vein blood from 191 normal adults, 167 males and 24 females. "Acceptable data" here is taken to mean results from

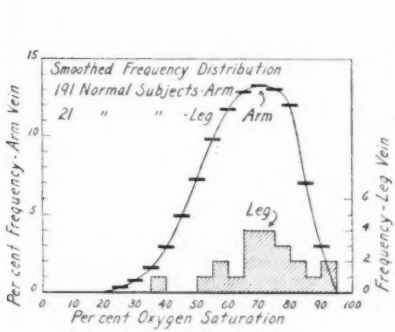



Fig. 5

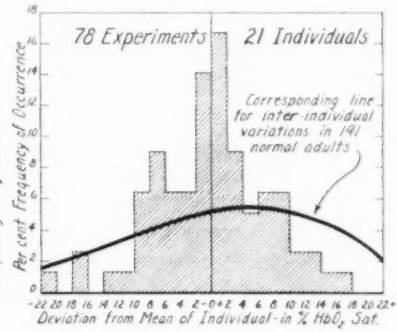

78 Experiments
21 Individuals
Corresponding line for inter-individual variations in 191 normal adults
Per cent Frequency of Occurrence
Deviation from Mean of Individual - in % HbO2 Sat.

Fig. 6

Fig. 5. Distribution of oxygen saturations in venous blood from the arm and from the leg in the resting state. Leg blood drawn from the femoral vein.

Fig. 6. Intra-individual variations in the oxygen saturation of venous blood from the antecubital fossa in normal adults in the resting state (60 per cent of these cases in the basal state). The results are plotted in terms of deviations, in per cent oxygen saturation, from the mean of each individual. The corresponding line for inter-individual variations is given for comparison. This line is a smoothed curve drawn through the deviations for 191 individuals, in steps of 2 per cent oxygen saturation, from the mean of all these individuals.

studies in which standard methods were used under reasonably controlled conditions. Only relatively long series were included, partly to save labor but more particularly because criteria for judging acceptability are generally absent from papers which present only a few incidental analyses. The sources of the data summarized in figure 5, together with the mean values for the several series, are given in table 2.

It will be noted that the mean values in table 2 are closely concordant except for the series of Cullen and Robinson. The blood samples for the latter study were taken from medical students at the start of a laboratory period after an hour lecture; the conditions are undoubtedly the least acceptable of any of the series in table 2. A mean value similar to that of

Cullen and Robinson may be calculated from the data of Looney and Jellinek (1937), if a standard normal oxygen capacity is assumed for their subjects (they did not measure oxygen capacity). As their subjects were men on relief, this assumption may be unjustifiable. Looney and Jellinek intimate that their low value for venous oxygen content is a result of the care taken to prevent entrance of oxygen into the blood after it was drawn; this implied criticism of other work cannot hold for the present series in which all blood samples were immediately transferred to the Van Slyke machine. Moreover, we cannot agree with their criticism of the common procedure of storing blood under oil for an hour or two before analysis. When heavy mineral oil is used in a layer 1 or 2 cm. thick over blood in a narrow tube at low temperature and when transfers are made through capillary tubes of 1 mm. bore, we consistently find only insignificant changes in the gas contents within the first two hours.

TABLE 2

*Sources of data on normal oxygen saturation in venous blood from the arm, together with numbers of subjects and mean values*

| AUTHORITY | NUMBER OF SUBJECTS | MEAN VALUE, SAT. | COMMENT |
|---|---|---|---|
| | | *per cent* | |
| Lundsgaard (1918) | 12 | 67.7 | Varying rest period |
| Lundsgaard and Möller (1923) | 17 | 76.1 | 29 experiments |
| Cullen and Robinson (1923) | 23 | 53.1 | Inadequate rest |
| Goldschmidt and Light (1925) | 50 | 70.0 | Some stasis |
| Weiss and Ellis (1935) | 17 | 68.5 | Ambulatory patients |
| Present series | 72 | 68.4 | |
| Grand mean | | 68.1 | (weighted) |

The variations in venous oxygen saturation presented in this paper may be thought to reflect individual characteristics in circulatory habitus. This is only partly true; intra-individual variation accounts for a considerable part of the total variation observed. This is shown by figure 6 in which are plotted the intra-individual variations, as deviations from the mean for each of the individuals, in 78 experiments on 21 individuals. The data plotted in figure 6 are derived from 3 sources: 1, twenty observations on a single individual given by Lundsgaard (1918); 2, seventeen observations on 5 individuals given by Lundsgaard and Möller (1923), and 3, forty-one observations on 15 individuals in the present series. In all cases repeated observations on a given individual were made in the resting state on different days. For comparison I have plotted the corresponding line for the variations from the mean for the 191 individuals summarized in figure 5, recalculated for steps of 2 per cent in oxygen saturation.

From figure 6 it will be seen that 50 per cent of all observations deviate more than ±10 per cent $HbO_2$ saturation from the mean of all the individuals and that intra-individual variation accounts for at least a third of these more variant observations. Outside the range ±6 per cent of the mean $HbO_2$ saturation are found 69.5 per cent of all observations in all individuals; outside the range ±6 per cent of the mean $HbO_2$ saturation for the individual are found 42.3 per cent of all observations on the individual. It should be noted that the intra-individual variations are undoubtedly underestimated owing to the small series of observations made on each individual.

Discussion. There is no doubt that the oxygen saturation of blood from veins in the antecubital fossa of the arm in normal human subjects shows a great range of variation, even when the external and physiological conditions are maintained as constant as is practicable. The very limited clinical utility of measurement of oxygen saturation of arm vein blood should, therefore, be apparent. Similarly, evaluation, from the saturation of arm vein blood, of the effect of experimental procedures can only be made on the basis of a statistically adequate series of measurements.

So far as the data go, the saturation of the blood from the great veins of the legs seems to be fully as variable as that from the arm. This being the case, it appears that the blood draining tissues representing more than a third of the mass of the body is highly variable in its oxygen saturation when the subject is at rest under constant conditions. This means, since the metabolism under these conditions is relatively constant, that the rate of circulation through these tissues must be highly variable.

This variability is in sharp contrast with the reported constancy of the total circulation of the body of normal subjects in basal rest (Grollman, 1932). Grollman reports (op. cit., p. 87) an arterio-venous oxygen difference which only varied from 5.5 to 6.7 cc. of oxygen per 100 cc. of blood for the total circulation in 50 normal adults. For his normals he reported an average deviation from the mean (5.9 cc. per 100 cc.) of only 4.4 per cent. However, Christensen's (1931) measurements on resting normals show considerably greater variation, in spite of the care with which his work was done.

In order to bring the present observations into harmony with Grollman's report it is necessary to conclude that variations in the blood flow through the limbs are counterbalanced by opposite variations in the blood flow through the rest of the body and that these adjustments result in a high degree of constancy of total blood flow not only in a given individual but in all normal adults at rest.

It may be objected that the fraction of the circulation representing blood flow through the arms and legs at rest is less than would be indicated by the mere relative bulk of the tissues. Data on this point are provided in measurements of the circulation rate in the hands from the calorimetric

method (Stewart, 1911) and by the plethysmographic method (Hewlett and Van Zwaluwenberg, 1913; Freeman, 1935). These measurements show very large intra- and inter-individual variations, ranging from 1 to 15 cc. per minute per 100 cc. of hand, but about 6 cc. represents a fair average for the hand at a relatively normal skin temperature. This is exactly the same as Grollman's average figure for the total circulation rate—60 cc. per minute per kilogram of body weight.

It is of interest that Grollman's mean value for the arterio-venous oxygen difference is not much greater than the average for the difference in both the arm and leg bloods recorded here. In the present study the mean arterio-venous difference in the arm was 5.2 cc. of oxygen per 100 cc. of blood, and 4.2 cc. in the leg (mean of the 21 cases given in fig. 5). Weiss and Ellis (1935) recorded the same average arterio-venous difference, 4.7 cc. of oxygen per 100 cc. of blood, in both the arms and the legs of their 17 "controls" (ambulatory patients). Under these resting conditions it appears that the oxygen metabolism of the extremities, per unit weight, is not much less than that for the rest of the body.

## SUMMARY

A study was made of the oxygen saturation of the blood from the arm veins, under carefully controlled conditions, on 59 normal men and 24 normal women, between the ages of 18 and 50.

Sixty-three subjects in basal rest had an average saturation of the arm vein blood of 68.2 per cent and a range of 25 to 85 per cent. Thirty per cent of the observations were outside the range 60 to 85 per cent which is frequently stated to be the "normal limit." These variations are not related to variations in the oxygen capacity of the blood of the individuals.

Compilation of data from other sources for normal adults in the "resting" condition shows similar individual variations. For 191 resting normal adults the range in oxygen saturation in the arm veins was from 24.5 per cent to 93.0 per cent, with an average of 68.1 per cent.

For 21 resting normal men the oxygen saturation in blood from the femoral vein ranged from 38.4 to 91.2 per cent, with an average of 71.2 per cent. Thirty-three per cent of the observations were outside the range 60 to 85 per cent of saturation.

The effect of a light breakfast on the oxygen saturation of blood from the arm is to produce only random alterations averaging $\pm 5.4$ per cent.

Brief but profound disturbance to the circulation was produced by maximal exercise for 1 minute and by the intravenous injection of epinephrine to bring the blood pressure momentarily to about 200 mm. Hg. One hour later the oxygen saturation of the arm vein blood was generally somewhat lower than in basal rest but there was a marked correlation between the two values in different individuals.

In 78 experiments on 21 individuals it was found that intra-individual variations account for a large share of the total variation.

The per cent oxygen saturation of the arm vein blood in normal women tends to be slightly lower and the oxygen yielded to the tissues, per 100 cc. of blood, tends to be slightly greater than in normal men in the basal state.

Calculations are presented to show that in order to harmonize with Grollman's values for the cardiac output it is necessary to assume that, in the basal state, there must be large and opposite variations in the circulation in the head and torso such as to produce the reported constancy of the total circulation.

## REFERENCES

(1) Christensen, E. H. Arbeitsphysiologie **5:** 18, 1931.
(2) Cullen, G. E. and H. W. Robinson. J. Biol. Chem. **57:** 533, 1923.
(3) Florkin, M., H. T. Edwards and D. B. Dill. This Journal **94:** 459, 1930.
(4) Freeman, N. E. This Journal **113:** 384, 1935.
(5) Goldschmidt, S. and A. B. Light. This Journal **73:** 127, 1925.
(6) Grollman, A. The cardiac output of man in health and disease. Baltimore, C. C. Thomas, 1932.
(7) Hewlett, A. W. and J. G. Van Zwaluwenburg. Arch. Int. Med. **12:** 1, 1913.
(8) Hewlett, A. W., J. G. Van Zwaluwenburg and J. H. Agnew. Arch. Int. Med. **12:** 7, 1913.
(9) Keys, A. and A. M. Snell. J. Clin. Investigation **17:** 59, 1938.
(10) Looney, J. M. and E. M. Jellinek. This Journal **118:** 225, 1937.
(11) Lundsgaard, C. J. Biol. Chem. **33:** 133, 1918.
(12) Lundsgaard, C. and E. Möller. J. Biol. Chem. **55:** 315, 1923.
(13) Peters, J. P. and D. D. Van Slyke. Quantitative clinical chemistry. I. Interpretations. p. 570. Baltimore, Williams and Wilkins, 1931.
(14) Stewart, G. N. Heart **3:** 33, 1911.
(15) Stewart, G. N. Heart **3:** 76, 1911.
(16) Theis, F. V. and M. R. Freeland. J. A. M. A. **107:** 1907, 1936.
(17) Weiss, S. and L. B. Ellis. Arch. Int. Med. **56:** 665, 1935.

# A STUDY OF THE CIRCULATORY FAILURE AND SHOCK FOLLOWING TRAUMA TO THE HEALTHY, VIGOROUS ADRENALECTOMIZED DOG

W. W. SWINGLE, W. M. PARKINS, A. R. TAYLOR[1] AND H. W. HAYS

*From the Biological Laboratory, Princeton University, Princeton, N. J.*

Received for publication June 8, 1938

A fundamental difference exists between intact and adrenalectomized dogs with respect to their susceptibility to identical shock inducing procedures. The animal with adrenal glands is highly resistant to the procedures usually employed for producing shock experimentally, and maintains an adequate circulation despite severe tissue abuse. On the other hand, the dog lacking these glands, but receiving daily injections of sufficient hormone to maintain normal health and vigor, promptly develops shock and circulatory collapse in response to any untoward stimulus which throws a strain upon the organism. Practically every investigator who has used adrenalectomized dogs has commented upon this fact, and two of the present writers have discussed the problem in some detail (Swingle and Parkins, 1935).

This type of animal affords ideal material for study of circulatory failure since the degree of trauma necessary to induce shock is not sufficient to injure the tissues or organs seriously or mutilate the dog. The dogs can be revived from shock and restored to normal health without difficulty if treated with adequate amounts of cortical hormone. A direct pressor action of the hormone upon the circulation becomes evident during recovery.

METHODS AND TYPE OF ANIMAL EMPLOYED. Thirteen strong, vigorous male dogs, bilaterally adrenalectomized for periods ranging from six to twelve months were used. Seven were used for intestinal stripping and six for muscle trauma. They were maintained in normal health by daily injections of cortical hormone. All of the animals had at some time been studied through cycles of adrenal insufficiency and recovery in connection with other experiments. Study of this type of animal in comparison with intact dogs kept under similar laboratory conditions has convinced us that they are normal, healthy animals as judged by all criteria known to us for detecting signs or symptoms of inadequate hormone dosages or insufficiency. The dogs are active, vigorous, eat full rations, and are

[1] E. R. Squibb & Sons Fellow in the Biological Sciences.

at peak weight; we frequently use them for breeding purposes. The blood pressure, pulse, serum electrolytes, hemoconcentration, blood sugar and blood urea nitrogen are normal. Study of the water balance and renal excretion of electrolytes also shows them to be normal.

The direct needle puncture blood pressure method used to determine mean arterial pressure has been described (Parkins, 1934). The animals were trained to remain quiet and relaxed during the determinations; frequently they appeared to go to sleep on the table. The normal resting pressure of equally well trained intact dogs does not differ essentially from that of the healthy vigorous adrenalectomized animal receiving adequate maintenance dosages of hormone (Parkins, 1934).

Two methods commonly used in the study of experimental shock, i.e., muscle trauma and intestinal manipulation, have been employed to induce circulatory collapse. The adequacy of these methods and their application to the study of experimental shock in the intact animal have been discussed by numerous authors. *1. Exposure and manipulation of the intestines:* Mann, 1914 and 1919–20, 1935; Wiggers, 1917–18; Gasser, Erlanger and Meek, 1919–20; Blalock, 1931; Beard and Blalock, 1932; Roome, Keith and Phemister, 1933. *2. Trauma to muscle masses:* Bayliss and Cannon, 1919; Cannon, 1923; Aub, 1920–21; Blalock, 1930; Parsons and Phemister, 1930; Bell, Clark and Cuthbertson, 1938; Johnson and Blalock, 1931; Roome, Keith and Phemister, 1933, and O'Shaughnessy and Slome, 1934–35.

In those experiments involving intestinal stripping, the dogs were anesthetized with ether, and using aseptic precautions, a three-inch incision was made through the mid-line into the body cavity. A small loop of intestine was brought to the exterior and stripped through the fingers and the stripping continued along the intestine using small segments at a time. Very little gut was exposed during the procedure. This was continued for periods varying in the different dogs from twenty to thirty minutes (table 1). The wound was then sutured and bandaged and the animal permitted to recover from the anesthetic.

Muscle trauma was effected by blows from a wooden mallet upon the thigh muscles of one hind limb. The animals were under deep ether anesthesia and the degree of trauma was quite insufficient to more than severely bruise the muscles.

The various methods utilized for study of the blood constituents have been referred to repeatedly in earlier papers of this series of adrenal studies (Swingle, Parkins, Taylor and Hays, 1937).

*Serum electrolytes.* There are no significant changes in the concentration of the extracellular electrolytes, Na and Cl, in the shock produced by stripping the intestine, or trauma to muscle masses. These findings are in agreement with those reported by us for shock induced by injecting

massive doses of adrenalin (Parkins, Swingle, Taylor and Hays, 1938). Since the levels of the serum Na and Cl remain unchanged even when the

TABLE 1

*Blood pressure, electrolyte and hemoconcentration changes in healthy adrenalectomized dogs shocked by intestinal manipulation*

| DATE | TIME | BLOOD PRESSURE | PULSE PER MINUTE | SERUM SODIUM | SERUM CHLORIDE | SERUM POTASSIUM | HEMOGLOBIN | RED CELL VOLUME | R.B.C. | STRIPPING INTERVAL | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | #1 ♂—10.5 kgm. | | | | | | | |
| | *hours* | *mm. Hg* | | *m.-eq. per liter* | *m.-eq. per liter* | *m.-eq. per liter* | *grams per cent* | *per cent* | *millions* | *minutes* | |
| 9/23/37 | 10:20 a.m. | 101 | 84 | 141.6 | 109.8 | 5.9 | 10.8 | 35.1 | 6.24 | 23 | Normal, int. strip |
| 9/23/37 | 2:20 p.m. | 88 | 176 | | | | | | | | |
| 9/23/37 | 4:20 p.m. | 67 | 160 | | | | | | | | |
| 9/23/37 | 6:00 p.m. | 42 | 148 | 145.0 | 112.6 | 5.5 | 11.3 | 38.0 | 6.62 | | Shock, extract* |
| 9/24/37 | 10:00 a.m. | 84 | 112 | | | | | | | | |
| | 10:00 p.m. | 92 | 100 | | | | | | | | |
| 9/25/37 | 11:00 a.m. | 106 | 88 | 145.5 | 115.2 | 5.4 | 10.5 | 35.2 | 5.31 | | Normal |
| | | | | #2 ♂—11.6 kgm. | | | | | | | |
| 9/17/37 | 10:20 a.m. | 106 | 96 | 144.6 | 113.2 | 6.2 | 12.5 | 39.5 | 5.84 | 20 | Normal, int. strip |
| 9/17/37 | 11:30 a.m. | 86 | 180 | | | | | | | | |
| 9/17/37 | 2:30 p.m. | 63 | 176 | | | | | | | | |
| 9/17/37 | 5:00 p.m. | 48 | 168 | 141.2 | 109.8 | 5.3 | 17.3 | 48.2 | 7.93 | | Shock, extract* |
| 9/18/37 | 11:30 a.m. | 83 | 160 | | | | | | | | |
| 9/19/37 | 11:30 a.m. | 102 | 92 | 137.9 | 105.8 | 4.2 | 12.8 | 39.8 | 5.92 | | Recovery |
| | | | | #4 ♂—9.3 kgm. | | | | | | | |
| 9/30/37 | 9:35 a.m. | 108 | 80 | 141.9 | 114.8 | 5.7 | 10.2 | 29.8 | 4.26 | 22 | Normal, int. strip |
| 9/30/37 | 4:00 p.m. | 82 | 200 | | | | | | | | |
| 9/30/37 | 6:10 p.m. | 52 | 196 | 140.3 | 114.8 | 4.9 | 11.4 | 33.0 | 4.27 | | Shock, extract* |
| 10/ 1/37 | 9:20 a.m. | 90 | 140 | | | | | | | | |
| 10/ 2/37 | 9:00 a.m. | 116 | 76 | 141.4 | 115.0 | 4.5 | 9.9 | 33.7 | 3.88 | | Recovery |
| | | | | #6 ♂—13.0 kgm. | | | | | | | |
| 10/12/37 | 9:55 a.m. | 100 | 88 | 140.5 | 113.8 | 6.3 | 14.8 | 46.3 | 6.68 | 24 | Normal, int. strip |
| 10/12/37 | 12:00 m. | 88 | 148 | | | | | | | | |
| 10/12/37 | 2:30 p.m. | 76 | 144 | | | | | | | | |
| 10/12/37 | 6:20 p.m. | 40 | 112 | 142.2 | 115.8 | 5.5 | 18.5 | 59.0 | 8.62 | | Shock, discontinued* |
| | | | | #7 ♂—10.4 kgm. | | | | | | | |
| 5/16/38 | 10:10 a.m. | 108 | 84 | 141.6 | 105.8 | 5.7 | 10.8 | 32.9 | 4.80 | 30 | Normal, int. strip |
| 5/16/38 | 12:30 p.m. | 82 | 200 | | | | | | | | |
| 5/16/38 | 2:35 p.m. | 74 | 224 | | | | | | | | |
| 5/16/38 | 4:40 p.m. | 42 | 180 | 140.7 | 113.4 | 6.8 | 15.1 | 55.2 | 7.44 | | Shock.† Death 2 hrs. later |

* Three cubic centimeters cortical extract per kilogram body weight injected intravenously, when in shock, and 3 cc. per kgm. intraperitoneally in divided doses daily until recovery was complete.

† No treatment given.

animal is moribund, it is evident that the circulatory collapse is not due (as we had previously supposed) to disturbance of the extracellular electro-

lyte pattern with resulting upset in the internal distribution of body water between extracellular (including vascular) compartments and intercellular compartments. The hemoconcentration, when present, and loss of effective circulating fluid volume, are due to factors other than Na or Cl and associated fluid changes. This interpretation is contrary to an earlier view expressed by us on this subject (Parkins, Taylor and Swingle, 1935; Swingle and Parkins, 1935).

In uncomplicated adrenal insufficiency in the dog, the extracellular electrolytes, Na and Cl, are much reduced owing to increased renal elimination of these ions following cortical hormone withdrawal (Loeb et al., 1933; Harrop et al., 1933). However, if the adrenalectomized dog should become anuric soon after extract is discontinued, the serum electrolytes remain within the normal range, but nevertheless the circulation fails and the animal dies presenting all the signs and symptoms of adrenal insufficiency (Swingle, Parkins, Taylor and Hays, 1937). The circulatory failure in such cases is comparable to that induced by intestinal manipulation and muscle trauma and is not complicated by electrolyte changes or disturbance of the normal water balance of the body. Thus the probable reason why serum electrolyte changes do not appear in the shocked dog is because the time interval is so short and the animals are generally anuric until after they are injected with cortical hormone.

The serum K usually did not increase in the dogs subjected to intestinal stripping; in fact, the tendency was for this electrolyte to decline slightly when the animal was in collapse (table 1, animals 1, 2, 4 and 6). Obviously the circulatory failure and shock occurring in these dogs cannot be attributed to changes in serum K.

In general the same statement holds true for those animals shocked by muscle trauma. But two cases out of six exhibited significant increases in serum K (table 2, dogs 1 and 4). The samples were taken when the animals were in deep shock approximately twelve hours after traumatization. Apparently the time interval elapsing between trauma and collapse markedly influences the degree of K concentration. The longer the interval the greater the increase.

The evidence seems to indicate the changes in serum Na, Cl and K, are not important factors in shock insofar as the healthy, vigorous, adrenalectomized dog is concerned. We have pointed out in a previous publication that the fatally shocked normal dog does not show noteworthy alterations in serum Na and Cl.

*Hemoconcentration.* Hemoglobin, red cell volume and RBC were employed as indices of hemoconcentration changes. The results were inconstant and differ for each animal although in general some degree of concentration was present. We anticipated much greater blood concentration than actually occurred, in view of the consensus of opinion of students of experimental shock that hemoconcentration is invariable an

feature of the syndrome in intact animals. Under the conditions of the experiment, some shocked dogs of both the intestinal manipulation and muscle trauma series, revealed a degree of blood concentration little different from that present in adrenal insufficiency at a similar level of

TABLE 2

*Blood pressure, electrolyte and hemoconcentration changes in shock induced by muscle trauma*

| DATE | TIME | BLOOD PRESSURE | PULSE PER MINUTE | SERUM SODIUM | SERUM CHLORIDE | SERUM POTASSIUM | HEMOGLOBIN | RED CELL VOLUME | R.B.C. | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | #1 ♂—12.3 kgm. | | | | | | |
| | *hours* | *mm. Hg* | | *m.-eq. per liter* | *m.-eq. per liter* | *m.-eq. per liter* | *grams per cent* | *per cent* | *millions* | |
| 11/30/37 | 9:40 a.m. | 108 | 88 | 141.0 | 111.2 | 6.6 | 15.2 | 41.8 | 5.88 | Normal |
| 11/30/37 | 12:15 a.m. | 100 | 184 | | | | | | | Depressed |
| 11/30/37 | 6:15 p.m. | 82 | 190 | | | | | | | Lethargic |
| 11/30/37 | 9:30 p.m. | 38 | 176 | 141.6 | 116.8 | 11.1 | 20.5 | 48.6 | 7.40 | Shock.† Died 9:45 p.m. |
| | | | | #2 ♂—9.0 kgm. | | | | | | |
| 4/11/37 | 10:10 a.m. | 98 | 64 | 140.1 | 112.0 | 5.3 | 11.7 | 32.5 | 4.88 | Normal |
| 4/11/37 | 12:00 m. | 60 | 92 | | | | | | | Weak, listless |
| 4/11/37 | 2:00 p.m. | 58 | 132 | | | | | | | Weak, lethargic |
| 4/11/37 | 4:00 p.m. | 44 | 180 | 143.5 | 115.8 | 6.1 | 12.8 | 34.8 | 5.4 | Shock.† Died 7:30 p.m. |
| | | | | #3 ♂—13.2 kgm. | | | | | | |
| 5/12/38 | 10:00 a.m. | 102 | 84 | 142.7 | 113.6 | 5.5 | 11.7 | 36.7 | 5.0 | Normal |
| 5/12/38 | 12:00 m. | 66 | 140 | | | | | | | Listless |
| 5/12/38 | 2:10 p.m. | 58 | 144 | | | | | | | Lethargic |
| 5/12/38 | 5:15 p.m. | 52 | 140 | 144.6 | 115.8 | 6.3 | 12.6 | 38.6 | 7.44 | Shock.† Death 9:30 p.m. |
| | | | | #4 ♂—13.8 kgm. | | | | | | |
| 5/27/38 | 10:50 a.m. | 106 | 88 | 141.6 | 112.8 | 5.8 | 10.5 | 30.9 | 4.88 | Normal |
| 5/27/38 | 2:30 p.m. | 84 | 192 | | | | | | | Depressed |
| 5/27/38 | 6:30 p.m. | 68 | 196 | | | | | | | Lethargic |
| 5/27/38 | 9:30 p.m. | 52 | 208 | 139.2 | 110.2 | 10.2 | 13.8 | 40.8 | 7.20 | Shock, weak* |
| | | | | #5 ♂—13.6 kgm. | | | | | | |
| 5/27/38 | 10:00 a.m. | 108 | 92 | 140.8 | 110.8 | 5.6 | 12.4 | 39.8 | 6.20 | Normal |
| 5/27/38 | 12:35 a.m. | 80 | 140 | | | | | | | Listless |
| 5/27/38 | 2:35 p.m. | 68 | 132 | | | | | | | Lethargic |
| 5/27/38 | 5:00 p.m. | 50 | 128 | 140.0 | 113.8 | 5.8 | 14.3 | 45.2 | 6.80 | Shock* |

* Dogs 4 and 5 were injected with cortical extract (3 cc./kgm. per day) until recovery was complete.
† No treatment given.

blood pressure (dogs 2, 6, and 7, table 1 and dog 1, table 2). On the other hand the hemoconcentration was neglibible in several of the animals even when they were in collapse and at the point of death (dogs 1 and 4, table 1 and dogs 2 and 3, table 2). It is apparent that in the type of animal employed in these experiments, it is possible to have an animal die from

circulatory collapse with but scanty evidence for any accompanying hemoconcentration.

Hemoconcentration, when present, is apparently due to loss of vascular fluid owing to capillary stasis and dilatation with resulting increased permeability. We do not attribute the increased permeability of the capillaries directly to lack of cortical hormone; rather, we assume, that it is due to the general capillary atony, dilatation and stasis which results from lack of hormone.

*Blood pressure changes and the pressor action of cortical hormone in shock.* The arterial pressures recorded in the tables are mean pressures as determined in the femoral artery of the unanesthetized animal. The initial pressures recorded represent normal resting pressures taken ten minutes before ether was administered. The second reading was taken at various intervals after the animal had recovered from the anesthetic. In those cases revived from shock and restored to normal health by cortical hormone, the hormone was injected when the dog was in profound shock with a falling blood pressure. During the course of an extensive experience with healthy, vigorous adrenalectomized animals subjected to intestinal manipulation and muscle trauma, we have not observed these animals to spontaneously revive from shock and prostration following these procedures. In the absence of adequate cortical hormone, these animals seem to lack ability to raise the blood pressure spontaneously once it has fallen to a low level. They differ in this respect from the intact animal which apparently experiences no difficulty in making capacity adjustments at almost any pressure level (Swingle and Parkins, 1935).

As shown in tables 1 and 2, intestinal stripping and muscle trauma rapidly induces fatal secondary shock. The degree of trauma as administered to these animals fails to elicit any sign or symptom of shock or circulatory failure in the dog with intact adrenals. It requires prolonged intestinal stripping and a surprising amount of muscle trauma, even necessitating crushing of leg muscle and bones, to lower the arterial pressure of the intact dog to 50–40 mm. Hg and maintain it at this low level until complete circulatory collapse occurs.

The striking effect of cortical hormone in reviving the animals from shock and restoring the circulation is shown in the tables.

*Effect of hypertonic saline upon the low blood pressure in shock induced by intestinal manipulation and muscle trauma.* Hypertonic saline representing 20 cc. of a 20 per cent solution injected slowly at the rate of 1 cc. per minute through the jugular vein when the animal is in shock (arterial pressure 50 mm. Hg) raised the pressure to normal levels within twenty minutes. The gross symptoms of shock disappeared. Shortly following completion of the injection, the blood pressure slowly fell and within a few hours was again at shock level. Administration of cortical hormone

was necessary to restore the animal to normal. The temporary restorative effect of hypertonic solutions upon the circulation in the shocked intact dog is well known (Mann, 1919–20; Gasser, Erlanger and Meek, 1919–20; Erlanger and Gasser, 1919–20).

Discussion. The circulatory collapse following mild trauma to the intestine and to muscle masses of the hind limb of the healthy, vigorous adrenalectomized dog receiving maintenance doses of cortical hormone, is essentially unrelated to changes in serum electrolytes. Significant hemoconcentration may or may not be evident. It seems clear that upsets in distribution of body water and shift of fluids from extracellular to intracellular compartments is not an important factor. The degree of trauma used in these experiments was insufficient to produce great local losses of fluid into the area of injury although some local loss did occur in each set of experiments. However, it is doubtful if such local losses were sufficient to account for the diminution in effective volume of circulating fluid and circulatory collapse.

The marked slowing of the circulation and progressive fall in arterial pressure in the shocked dogs was associated with visceral congestion and numerous capillary hemorrhages indicative of peripheral vascular failure. It seems not unlikely that the circulatory failure and shock in these animals is primarily due to capillary atony, with resulting dilatation, stasis, and eventually increased permeability. The cause of death in both adrenal insufficiency and the shocked dog lacking adrenal glands is apparently due to the same thing—lack of sufficient cortical hormone to maintain the normal tonus of the capillaries.

The vigorous adrenalectomized dog on maintenance doses of hormone lacks sufficient hormone reserves to maintain capillary tone, when any stress or strain is thrown upon the circulation. Hence circulatory shock develops in response to mild trauma of any kind. When the capillaries lose tone a huge peripheral reservoir slowly opens up into which the blood flows and stagnates. The speed of blood flow is greatly reduced and the effective volume of circulating fluid sufficiently lowered to cause the circulation to fail.

In an earlier paper (Swingle and Parkins, 1935) attention was called to the fact that shock and circulatory collapse could be prevented in the vigorous adrenalectomized dog by priming the animal with large doses of cortical hormone previous to traumatization. By this procedure, according to our present interpretation, sufficient hormone reserves are made available to tide the animal over the crisis by enabling the dog to maintain normal peripheral vascular tone despite the trauma.

## SUMMARY

1. The shock and circulatory collapse induced by intestinal manipulation, and trauma to muscles of the healthy, vigorous adrenalectomized

dog on maintenance doses of hormones, are not due to changes in serum Na, Cl or K and associated disturbances in the distribution of body water.

2. Hemoconcentration, although usually present to some degree, is not an invariable accompaniment of shock and circulatory collapse in the type of animal used in these experiments.

3. Hypertonic saline given intravenously to the dog in profound shock, raises the arterial pressure to normal levels. The effect is temporary and the animal soon lapses into shock.

4. Adrenal cortical hormone when administered in adequate amounts restores the collapsed circulation to normal. The marked pressor effect of the hormone is apparently due to its regulatory control of capillary tone.

## REFERENCES


AUB, J. C. This Journal **44:** 388, 1920-21.

BAYLISS, W. M. AND W. B. CANNON. Report of Shock Committee, British Med. Research Committee, **25:** 1919.

BEARD, J. W. AND A. BLALOCK. J. Clin. Investigation **11:** 249, 1932.

BELL, J. R., A. M. CLARK AND D. P. CUTHBERTSON. J. Physiol. **92:** 361, 1938.

BLALOCK, A. Arch. Surg. **20:** 959, 1930.
Arch. Surg. **22:** 314, 598, 1931.

CANNON, W. B. Traumatic shock. D. Appleton & Co., New York, 1923.

ERLANGER, J. AND H. S. GASSER. This Journal **50:** 119, 1919-20.

GASSER, H. S., J. ERLANGER AND W. J. MEEK. This Journal **50:** 31, 1919-20.

GASSER, H. S. AND J. ERLANGER. This Journal **50:** 104, 1919-20.

HARROP, G. A., L. J. SAFFER, R. ELLSWORTH AND J. H. TRESCHER. J. Exper. Med. **58:** 17, 1933.

JOHNSON, G. S. AND A. BLALOCK. Arch. Surg. **23:** 855, 1931.

LOEB, R. F., D. W. ATCHLEY, E. M. BENEDICT AND H. LELAND. J. Exper. Med. **57:** 775, 1933.

MANN, F. C. Bull. Johns Hopkins Hosp. **25:** 205, 1914.
This Journal **50:** 86, 1919-20.
Am. J. Surg. N.S. **28:** 160, 1935.

O'SHAUGHNESSY, L. AND D. SLOME. Brit. J. Surg. **22:** 589, 1934-35.

PARKINS, W. M. This Journal **107:** 518, 1934.

PARKINS, W. M., A. R. TAYLOR AND W. W. SWINGLE. This Journal **112:** 581, 1935.

PARKINS, W. M., W. W. SWINGLE, A. R. TAYLOR AND H. M. HAYS. Proc. Soc. Exper. Biol. and Med. **37:** 675, 1938.

PARSONS, E. AND D. B. PHEMISTER. Surg. Gynec. and Obst. **51:** 196, 1930.

ROOME, N. W., W. S. KEITH AND D. B. PHEMISTER. Surg. Gynec. and Obst. **56:** 161, 1933.

SWINGLE, W. W. AND W. M. PARKINS. This Journal **111:** 426, 1935.

SWINGLE, W. W., W. M. PARKINS, A. R. TAYLOR AND H. W. HAYS. This Journal **119:** 684, 1937.

WIGGERS, C. J. This Journal **45:** 435, 1917-18.

# THE INFLUENCE OF CLOTHING ON THE PHYSIOLOGICAL REACTIONS OF THE HUMAN BODY TO VARYING ENVIRONMENTAL TEMPERATURES

A. P. GAGGE, C.-E. A. WINSLOW AND L. P. HERRINGTON

*Contribution no. 22, from the John B. Pierce Laboratory of Hygiene, New Haven, Conn.*

Received for publication June 11, 1938

In previous contributions from this laboratory (Winslow, Herrington, and Gagge, 1936a, b; 1937a, b; Gagge, 1936; Gagge, Herrington, and Winslow, 1937; Herrington, Winslow, and Gagge, 1937), we have presented the results of a series of studies of thermal interchanges between the human body and its environment, made by a new procedure which we have called partitional calorimetry.

In these experiments the subject is placed in a semi-reclining position in a copper booth. The reflecting power of the copper for radiant energy permits the maintenance of a wide differential between radiation and convection effects. Radiant heat of any desired intensity is reflected by the copper walls from heaters directed toward these walls from the outside through openings in the booth. Temperature and humidity of air within the booth are controlled by a highly sensitive air-conditioning system.

By techniques described in our earlier papers it is possible, for any given subject and for any given set of environmental conditions, to make a complete partition of thermal interchanges between the body and its environment in terms of the rate of metabolism ($M$), the rate of heat loss due to evaporation ($E$), the rate of heat loss by convection to, or gain from, the ambient air ($C$), the rate of heat loss by radiation to, or gain from, the surrounding walls ($R$), and the rate of heating and cooling of the body tissues.[1] The methods of determining these five factors are described in the papers cited above.

All studies heretofore published have been made with unclothed subjects. The present paper deals with the results of 312 separate experiments made with clothed subjects, but with all other procedures carried out in the same fashion described in earlier communications. The standard clothing employed consisted of a two-piece suit of cotton underwear, a cotton shirt without tie, socks, low leather shoes, and a dark gray single-ply suit with three-quarter lined coat and fully lined vest.

[1] The rate of cooling and the rate of heating of the body tissues is denoted by us as "Storage" ($S$). Positive storage is associated with a cooling process, negative storage with a heating process.

Measurements of the surface temperature of the clothed body were made by the Hardy radiometric method (Hardy, 1934) at the same 15 points used in our earlier studies (Winslow, Herrington, and Gagge, 1936a). Four of these points (three on the head, and one on the hand) were uncovered in the clothed subjects. For the other eleven points (on the forearm, the upper arm, and all points on the trunk and lower extremities), the radiometer record is that of the surface of the clothing, and the weighted means of all the 15 exposed points measured by the radiometer will be designated as clothing temperature ($T_{Cl}$). To obtain skin temperatures at the eleven points, covered by clothing, a rubberized cloth harness was devised to hold a set of copper buttons (to which were attached copper-constantan thermocouples) placed at appropriate points

TABLE 1

*Characteristics of subjects*

| SUBJECT | HEIGHT | WEIGHT | DUBOIS AREA | RADIATION AREA | CONVECTION CONSTANT ($k_C$) | RADIATION CONSTANT ($k_R$) | ENVIRONMENTAL CONSTANT ($k_O$) | PER CENT CONVECTION* | PER CENT RADIATION* |
|---|---|---|---|---|---|---|---|---|---|
| | *meters* | *kgm.* | *sq.m.* | *sq.m.* | *kgm. cal/hr.-°C.* | *kgm. cal/hr.-°C.* | *kgm. cal/hr.-°C.* | $k_C/k_O$ | $k_R/k_O$ |
| Clothed: | | | | | | | | | |
| VII | 1.88 | 79 | 1.92 | 1.63 | 6.84 | 8.82 | 15.66 | 44 | 56 |
| IX | 1.72 | 62.5 | 1.60 | 1.05 | 4.68 | 5.76 | 10.44 | 45 | 55 |
| Nude: | | | | | | | | | |
| VII | 1.88 | 79 | 1.92 | 1.63 | 10.08 | 8.82 | 18.90 | 53 | 47 |
| IX | 1.72 | 62.5 | 1.60 | 1.10 | 6.30 | 5.94 | 12.24 | 52 | 48 |
| I | 1.70 | 105. | 2.13 | 1.58 | 9.18 | 8.82 | 18.00 | 51 | 49 |
| II | 1.65 | 47.5 | 1.49 | 1.11 | 7.38 | 6.30 | 13.68 | 53 | 47 |

* Per cent of total loss by radiation plus convection where wall and air temperatures are the same and when air movement is 5 meters per minute.

against the skin. A weighted average of these 11 thermocouple readings and the four radiometer readings on the exposed surfaces represents the mean skin temperature ($T_S$).

In the present "clothed" experiments, two subjects were used (VII and IX); their physical characteristics are presented in table 1 for both nude and clothed conditions, while the subjects were in semi-reclining position. For comparison are added the physical characteristics of subjects I and II, which were used in our earlier "nude" experiments. For subjects VII and IX, these physical values, for both nude and clothed states, were derived experimentally by the Linearity Method described by us earlier (Gagge, 1936), the only difference being that for the clothed case the mean clothing temperature was used in the analysis instead of

the mean skin temperature. The radiation area was calculated on the assumption that the clothes acted as black bodies. On this assumption, as indicated in table 1, the radiation area for a given human body, whether nude or clothed, is essentially the same. One would ordinarily expect that the radiation area for the clothed subject would be slightly greater than when nude. This should be the case if the emissivity of the clothes were actually unity. However, whatever the emissivity may be, our technique of measuring the radiation area, in conjunction with Stefan's Radiation Law, must give correct values for the total radiation exchange. The convection constant ($k_C$) is valid only for the standard air movement of 17 feet per minute or approximately 5 meters per minute, used throughout the experiments reported here. It is interesting to note that the convection constant for the folded and rough surfaces of the clothing is distinctly less than the corresponding constant for the smoother skin surfaces of the nude, on account of interference with induced convection currents. The convection constant, $k_C$, for 5 meters per minute of air movement (like $A_R$) was determined by direct experiment.

In table 1 the term called "Radiation Constant," is derived from the Stefan Radiation Law, reduced to a first approximation. The Environmental Constant, $k_O$, is the sum of the radiation and convection constants. Thus, from the physical factors in table 1 it is possible at any time to estimate the Radiation Exchange, $R$, and Convection Loss, $C$, by the use of the following relations,

$$R = k_R (T_{Cl} - T_W), \tag{1}$$

and

$$C = k_C (T_{Cl} - T_A), \tag{2}$$

where $T_{Cl}$ is the mean surface temperature of body and clothing exposed to the environment. Adding (1) and (2), we have

$$R + C = k_O (T_{Cl} - T_O), \tag{3}$$

where $k_O$ equals the sum of $k_R$ and $k_C$, and the operative temperature, $T_O$, is defined by the relation

$$T_O = \frac{k_R T_W + k_C T_A}{k_O}, \tag{4}$$

and represents an average of the wall and air temperatures weighted according to the relative effectiveness of radiation and convection, as expressed by their constants indicated in table 1.

When the wall temperature is equal to the air temperature, the fraction or percentage of the total loss by radiation plus convection, which is given off as radiation or convection alone, is expressed by the ratio of $k_R$ or $k_C$ to $k_O$, respectively. These percentages are given in the last two columns of table 1. Here we note that the convection loss for the clothed subject

is lower than the loss by radiation. This is in striking contrast to the nude situation where the loss by convection is greater than the loss by radiation. It is interesting to note the general agreement of these percentages among the various subjects for both the nude and the clothed states.

Our values for per cent of radiation loss for clothed subjects at 5 meters per minute air velocity—55 to 56 per cent—are slightly below the 60 per cent value reported by Aldrich (1928) for "zero" air velocity.

*General thermal relations.* The experiments here reported were all conducted with a standard turbulent air velocity of 5 meters per minute and with a relative humidity of 40 to 50 per cent. Although air temperatures and wall temperatures were varied within wide limits, in the present paper our data are analyzed on the basis of operative temperature and of the calorie demand of the environment as expressed by $R + C$. The differential effects of radiation and convection will be discussed in a subsequent communication.

For the purpose of the present analysis, the fundamental data for 22 conditions for subject VII, and 27 conditions for subject IX, have been ordered in relation to operative temperature. Four-point moving averages were derived from these data for each of the two subjects. The averaged data for the two subjects combined are presented in table 2.

For comparison, there are presented in table 3 data for the nude subjects I and II, derived in a similar manner. In tables 2 and 3 the comfort vote, head temperature ($T_H$), temperature of the upper extremities ($T_U$), the trunk ($T_T$), the lower extremities ($T_L$), the rectal temperature ($T_B$), the clothing temperature ($T_{Cl}$), the metabolism ($M$), and the evaporation loss ($E$), represent values averaged directly from the basic data. The metabolism and evaporation loss are expressed in terms of unit area as measured by the Dubois Linear formula (Dubois and Dubois, 1916). The remaining columns in the tables are derived from these average values. The skin temperature, $T_S$, represents the average of $T_H$, $T_U$, $T_T$, and $T_L$, (weighted by the factors 7, 21, 31, and 41, respectively). The total loss by radiation and convection, i.e., the *calorie demand* of the environment, is derived from equation (3) above, using a mean value of 7.34 kgm. cal./m²-hr.-°C. for $k_O$, which follows directly from table 1 for subjects VII and IX, when clothed. For the nudes, subjects I and II, a mean value of 8.82 kgm.cal./m²-hr.-°C. for $k_O$ is used. The value of storage, $S$, has been chosen to satisfy the fundamental partition law

$$M + S = E + R + C$$

The column headed $K$, for conductance, is calculated from the basic relation derived in an earlier paper (Winslow, Herrington, and Gagge, 1937a) and described more fully later.

The generalized partitions for both clothed and nude subjects are pre-

TABLE 2*

*Averaged data—clothed subjects*

| $T_O$ | COMFORT VOTE | $T_H$ | $T_U$ | $T_T$ | $T_L$ | MEAN $T_S$ | $T_B$ | $T_{Cl}$ | $M$ | $S$ | $E$ | $R+C$ | $K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| °C. | | °C. | °C. | °C. | °C. | °C. | °C. | °C. | *kgm. cal./m²-hr.* | *kgm. cal./m²-hr.* | *kgm. cal./m²-hr.* | *kgm. cal./m²-hr.* | *kgm. cal. m²-hr.-°C.* |
| 38.9 | 4.1 | 37.2 | 35.9 | 36.5 | 35.8 | 36.2 | 37.4 | 36.9 | 50.0 | − .5 | 64.0 | −14.5 | 38.7 |
| 37.8 | 3.8 | 36.9 | 35.8 | 36.5 | 35.7 | 36.1 | 37.4 | 36.5 | 50.0 | 0 | 59.5 | −9.5 | 36.0 |
| 36.7 | 3.8 | 36.4 | 35.7 | 36.3 | 35.4 | 35.8 | 37.3 | 36.1 | 50.0 | −1.5 | 53.0 | −4.5 | 32.4 |
| 35.6 | 3.75 | 36.1 | 35.4 | 36.1 | 35.1 | 35.6 | 37.3 | 35.3 | 49.5 | −3.5 | 47.5 | −1.5 | 26.6 |
| 34.4 | 3.6 | 35.8 | 35.2 | 36.1 | 34.8 | 35.3 | 37.2 | 34.9 | 49.0 | −9.5 | 40.0 | − .5 | 20.9 |
| 33.3 | 3.5 | 35.4 | 35.0 | 35.9 | 34.7 | 35.2 | 37.2 | 34.4 | 48.0 | −4.0 | 36.0 | 8.0 | 21.4 |
| 32.2 | 3.1 | 35.3 | 34.8 | 35.6 | 34.1 | 34.8 | 37.2 | 34.0 | 46.5 | −3.0 | 30.5 | 13.0 | 18.2 |
| 31.1 | 2.8 | 35.3 | 34.6 | 35.2 | 33.6 | 34.4 | 37.1 | 33.6 | 46.5 | −1.0 | 27.0 | 18.5 | 17.5 |
| 30.0 | 2.3 | 34.8 | 34.1 | 35.1 | 33.1 | 34.1 | 37.0 | 33.1 | 46.5 | −3.0 | 21.0 | 22.5 | 14.8 |
| 28.9 | 2.0 | 34.5 | 33.6 | 34.8 | 32.6 | 33.6 | 36.9 | 32.1 | 47.0 | −6.5 | 16.5 | 24.0 | 12.3 |
| 27.8 | 1.85 | 34.3 | 33.2 | 34.9 | 32.3 | 33.4 | 36.9 | 31.7 | 48.5 | −5.5 | 14.5 | 28.5 | 12.3 |
| 26.7 | 1.9 | 34.1 | 32.3 | 34.8 | 31.5 | 32.9 | 36.9 | 30.8 | 49.5 | −4.0 | 15.0 | 30.5 | 11.2 |
| 25.6 | 2.2 | 33.8 | 31.8 | 34.6 | 31.1 | 32.6 | 36.9 | 30.3 | 50.0 | 0 | 15.0 | 35.0 | 11.4 |
| 24.4 | 2.4 | 33.6 | 31.9 | 34.5 | 30.3 | 32.2 | 36.9 | 29.7 | 50.0 | 3.5 | 15.0 | 38.5 | 11.4 |
| 23.3 | 2.6 | 33.3 | 30.9 | 34.3 | 29.7 | 31.6 | 36.9 | 29.0 | 49.5 | 7.0 | 15.0 | 41.5 | 10.6 |
| 22.2 | 2.8 | 32.9 | 30.4 | 34.2 | 29.1 | 31.2 | 36.9 | 28.4 | 49.0 | 10.5 | 14.0 | 45.5 | 10.4 |
| 21.1 | 3.1 | 32.8 | 30.0 | 34.1 | 28.7 | 30.9 | 36.9 | 27.8 | 48.0 | 15.0 | 14.0 | 49.0 | 10.4 |
| 20.0 | 3.2 | 32.4 | 29.4 | 33.9 | 28.4 | 30.6 | 36.9 | 27.2 | 49.5 | 16.5 | 13.0 | 53.0 | 10.4 |
| 18.9 | 3.15 | 32.1 | 29.1 | 33.9 | 28.2 | 30.4 | 36.9 | 27.0 | 50.5 | 21.5 | 12.0 | 60.0 | 11.0 |
| 17.8 | 3.2 | 31.7 | 28.6 | 33.8 | 27.8 | 30.1 | 37.0 | 26.6 | 51.0 | 25.0 | 11.0 | 65.0 | 11.0 |
| 16.7 | 3.5 | 31.3 | 27.6 | 33.6 | 26.9 | 29.4 | 36.9 | 25.6 | 52.0 | 24.5 | 11.0 | 65.5 | 10.3 |
| 15.6 | 3.75 | 30.8 | 26.9 | 33.3 | 26.3 | 28.9 | 36.9 | 24.7 | 53.0 | 25.0 | 11.0 | 67.0 | 9.7 |
| 14.4 | 3.9 | 30.4 | 26.3 | 33.0 | 26.3 | 28.7 | 36.9 | 23.9 | 54.5 | 26.0 | 10.5 | 70.0 | 9.7 |
| 13.3 | 3.8 | 30.2 | 26.4 | 32.9 | 26.3 | 28.6 | 36.8 | 23.8 | 55.0 | 32.5 | 10.5 | 77.0 | 10.6 |
| 12.2 | 3.85 | 29.8 | 26.2 | 32.7 | 26.1 | 28.4 | 36.8 | 23.4 | 55.0 | 37.0 | 10.0 | 82.0 | 11.0 |
| 11.1 | 4.0 | 29.3 | 25.7 | 32.6 | 25.6 | 28.1 | 36.8 | 22.7 | 56.5 | 38.5 | 10.0 | 85.0 | 10.8 |

* In tables 2 and 3 the basic observed data are: $T_O$, the operative temperature in °C.; $T_H$, the mean skin temperature of head in °C.; $T_U$, the mean skin temperature of upper extremities in °C.; $T_T$, the mean skin temperature of trunk in °C.; $T_L$, the mean skin temperature of lower extremities in °C.; $T_S$, the mean skin temperature found by averaging $T_H$, $T_U$, $T_T$, and $T_L$ weighted by the factors 7, 21, 31, and 41, respectively; $T_B$, the rectal temperature in °C.; $T_{Cl}$, the mean temperature of the exposed clothing and skin surfaces over the entire body in °C., weighted as in the case of $T_S$; $M$, the metabolism expressed in kgm.cal./m²-hr.; $E$, the total evaporation loss, expressed in kgm.cal./m²-hr. Comfort vote, expressed numerically by 1 (very pleasant), 2 (pleasant), 3 (indifferent), 4 (unpleasant), 5 (very unpleasant).

The derived data are: $R + C$, the total loss by radiation plus convection in kgm. cal./m²-hr., found by use of the environmental constants 7.34 kgm.cal./m²-hr.-°C. for the clothed, and 8.82 kgm.cal./m²-hr.-°C. for the nude. $S$, storage, or the rate of body cooling when positive, body heating when negative; the value of $S$ is so chosen that $M + S$ equals $E + R + C$. $K$, the conductance of the peripheral tissue, as defined by the relation $E + R + C/T_B - T_S$, and expressed in kgm.cal./m²-hr.-°C.

sented in figure 1. In the upper part of figure 2 are plotted the associated skin temperatures and clothing temperatures. For both cases the partitional factors are all plotted against $T_O$—the temperature operating on the exposed surface of the body system, whether it be skin or clothing, or both.

The effect of the addition of clothing is at once apparent, and the following differences may be noted.

TABLE 3*

*Averaged data—nude subjects*

| $T_O$ | COMFORT VOTE | $T_H$ | $T_U$ | $T_T$ | $T_L$ | MEAN $T_S$ | $T_B$ | $M$ | $S$ | $E$ | $R+C$ | $K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| °C. | | °C. | °C. | °C. | °C. | °C. | °C. | kgm. cal./m²-hr. | kgm. cal./m²-hr. | kgm. cal./m²-hr. | kgm. cal./m²-hr. | kgm. cal./m²-hr.-°C. |
| 38.9 | 3.5 | 35.7 | 34.7 | 35.2 | 35.1 | 35.1 | 37.1 | 49.5 | −2.0 | 81.0 | −33.5 | 23.8 |
| 37.8 | 3.2 | 35.5 | 34.7 | 35.1 | 34.8 | 34.9 | 37.1 | 49.0 | −2.0 | 72.0 | −25.0 | 21.8 |
| 36.7 | 2.6 | 35.2 | 34.6 | 35.1 | 34.6 | 34.8 | 37.1 | 49.0 | −7.5 | 58.0 | −16.5 | 18.2 |
| 35.6 | 2.4 | 35.2 | 34.6 | 35.0 | 34.4 | 34.7 | 37.0 | 49.0 | −8.0 | 48.5 | −7.5 | 18.0 |
| 34.4 | 2.5 | 34.9 | 34.6 | 34.9 | 34.5 | 34.7 | 37.0 | 49.5 | −4.5 | 43.0 | 2.0 | 19.3 |
| 33.3 | 2.5 | 34.9 | 34.6 | 34.9 | 34.4 | 34.7 | 36.9 | 51.5 | −3.5 | 36.0 | 12.0 | 21.6 |
| 32.2 | 2.4 | 34.8 | 34.7 | 34.9 | 33.8 | 34.4 | 36.8 | 49.0 | −3.0 | 26.0 | 20.0 | 19.3 |
| 31.1 | 2.2 | 34.3 | 34.4 | 34.8 | 33.3 | 34.1 | 36.7 | 48.0 | 1.0 | 23.0 | 26.0 | 20.2 |
| 30.0 | 2.1 | 33.7 | 34.1 | 34.4 | 32.7 | 33.6 | 36.7 | 49.0 | 4.0 | 21.0 | 32.0 | 17.1 |
| 28.9 | 1.95 | 33.6 | 33.7 | 33.9 | 32.3 | 33.2 | 36.7 | 49.5 | 6.5 | 18.0 | 38.0 | 15.9 |
| 27.8 | 2.0 | 33.1 | 33.3 | 33.6 | 31.6 | 32.7 | 36.6 | 49.5 | 9.5 | 16.0 | 43.0 | 15.0 |
| 26.7 | 2.6 | 32.3 | 31.9 | 32.7 | 30.4 | 31.6 | 36.5 | 48.5 | 11.0 | 16.5 | 43.0 | 12.1 |
| 25.6 | 3.0 | 31.3 | 31.4 | 32.3 | 29.7 | 30.9 | 36.6 | 48.0 | 16.0 | 16.0 | 48.0 | 11.4 |
| 24.4 | 2.9 | 32.0 | 31.1 | 32.1 | 29.1 | 30.6 | 36.6 | 46.0 | 24.5 | 16.0 | 54.5 | 11.9 |
| 23.3 | 2.7 | 32.0 | 31.1 | 31.9 | 28.9 | 30.6 | 36.6 | 47.0 | 32.0 | 15.0 | 64.0 | 13.2 |
| 22.2 | 3.6 | 30.4 | 29.8 | 31.5 | 28.3 | 29.7 | 36.6 | 48.0 | 31.5 | 13.5 | 66.0 | 11.7 |
| 21.1 | 4.1 | 29.8 | 29.4 | 30.9 | 27.4 | 29.1 | 36.6 | 46.5 | 37.0 | 13.0 | 70.5 | 11.2 |
| 20.0 | 4.2 | 29.4 | 29.1 | 30.6 | 27.3 | 28.8 | 36.5 | 47.0 | 34.0 | 13.0 | 78.0 | 10.6 |

* See note to table 2.

1. The metabolism remains essentially the same for the nude as well as the clothed subject in the range from 39°$T_O$ to 20°$T_O$. Below 20°$T_O$ there is a rise in metabolism.

2. The curve for evaporation loss for the nude subject is generally steeper at high operative temperatures, crossing the curve for the clothed subject at 33.5°$T_O$ and reaching its inflection point at about 31°$T_O$. The clothed curve, on the other hand, has a definite inflection point at 29°$T_O$.

3. The slope of the curve for heat interchange due to radiation plus convection is less steep for the clothed than for the nude subject. This is related to the lower environmental constant effective for the clothed

subject; and is also influenced by the greater drop in surface temperature of the clothing as compared with the skin temperature of the nude subject.

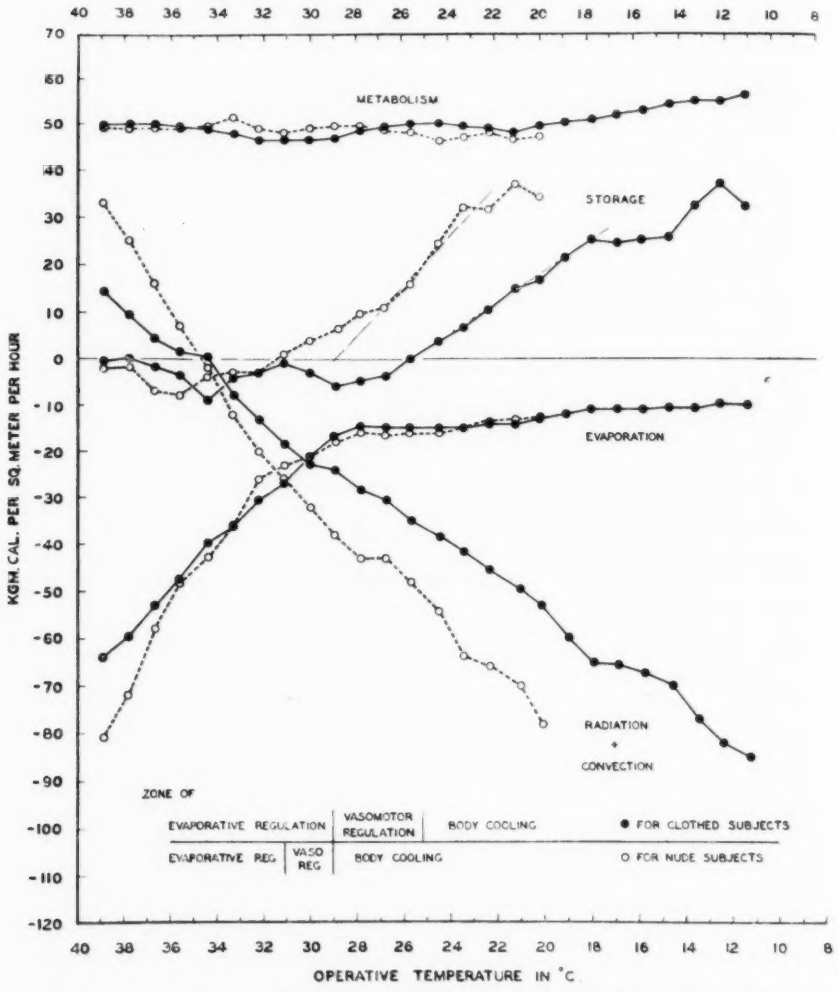



Fig. 1. Thermal interchanges for clothed and nude subjects at various operative temperatures.

4. The storage curve reflects the variation of the last two factors cited. Thus, the rate of body cooling for the clothed subject does not increase as much as the nude at lower operative temperatures. In other words,

the clothes offer protection for the body against cold, as would, of course, be expected. If the trend lines for storage, on the cold side, are continued

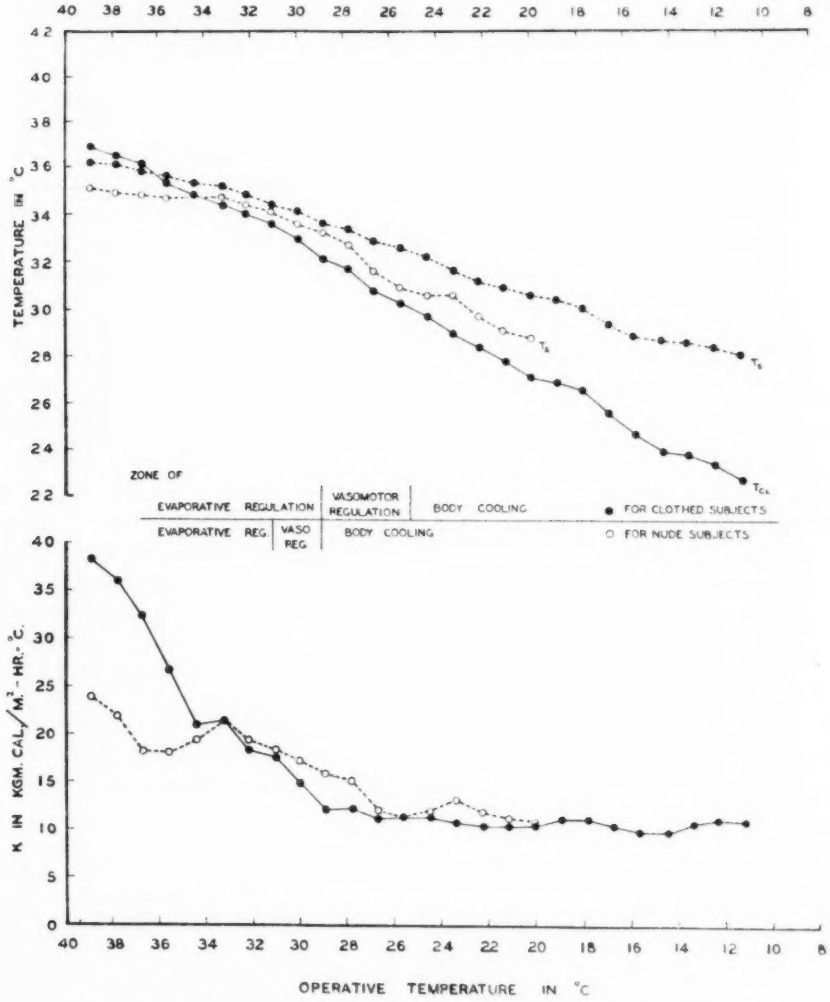



Fig. 2. Mean skin temperature and conductance for clothed and nude subjects for various operative temperatures.

through the zero ordinate, it will be seen that effective body cooling sets in at $29°T_O$ for the nude subject and at $25°T_O$ for the clothed subject.

5. In the operative temperature ranges between $31°T_O$ and $29°T_O$ for

the nude, and 29°$T_O$ and 25°$T_O$ for the clothed, neither evaporative regulation nor body cooling are important factors. In this range, thermal equilibrium is maintained primarily by vasomotor regulation. The range observed here for vasomotor control of the nude subject agrees well with that previously reported by Hardy and Dubois (1937, 1938).

6. The relation of clothing temperature to skin temperature (fig. 2) shows that clothing exerts a protective influence at both extremes of the temperature range. Below 25°$T_O$, since $T_{Cl}$ is less than $T_S$, clothing retards the loss of heat from the skin and decreases the rate of body cooling. Above 36°$T_O$, it retards the reception of heat from the hot environment. The change of clothing temperature with $T_O$ is essentially linear, but the skin temperature tends to level off at 33.5°$T_O$ for both clothed and nude subjects. This phenomenon is much more striking in the case of the nudes.

Rectal temperatures, as seen from tables 2 and 3, follow a parallel course, with the rectal temperatures always about a quarter of a degree higher for the clothed subjects on the hot side, the difference rising to about half a degree at certain points on the cold side. For both groups, the rectal temperatures decline with decreasing operative temperature down to a $T_O$ in the range 26.5°–29°C. Below this point the values hold nearly constant.

*The effect of clothing on the thermal gradient to environment.* The results as expressed in figures 1 and 2 demonstrate the protective value of the clothing in a qualitative way. It is important to measure this factor quantitatively. Considering the loss by radiation and convection alone, it is at once obvious that all heat passing the clothing-surface boundary must also pass the skin-surface boundary. Thus, the rate of heat loss in calories per hour from the skin surface must equal the corresponding loss from the clothing surface itself. If the temperature gradient from skin surface to clothing surface is known, one should be able to express in physical terms a quantitative measure of the conductance of the clothing. Thus, $K_{Cl}$, the added conductance due to the clothing, is defined by the equation,

$$K_{Cl} = \frac{R + C}{Ts - T_{Cl}}.$$

The above relation is true only if there is no evaporation taking place in the folds of the clothing. If we plot $R + C$ vs. $T_S - T_{Cl}$, we should get a straight line through the origin, provided this assumption holds. Such a plot is presented in figure 3. As the curve is colinear with the origin, it at once demonstrates that over the range of our experiments evaporation takes place essentially at the surface of the skin itself.[2] The value of the

[2] This conclusion, it should be noted, is based on experiments with low relative humidity and generally high radiation, giving a very low vapor pressure for $T_A$. If the difference between the vapor pressures of the skin and the ambient air were small, different relations might be manifest.

slope, 15.8 kgm.cal./m²-hr.-°C., measures the mean physical conductance of the clothing. In magnitude, this is slightly greater than the minimal conductance of the skin, namely, 11 kgm.cal./m²-hr.-°C. (see table 2).

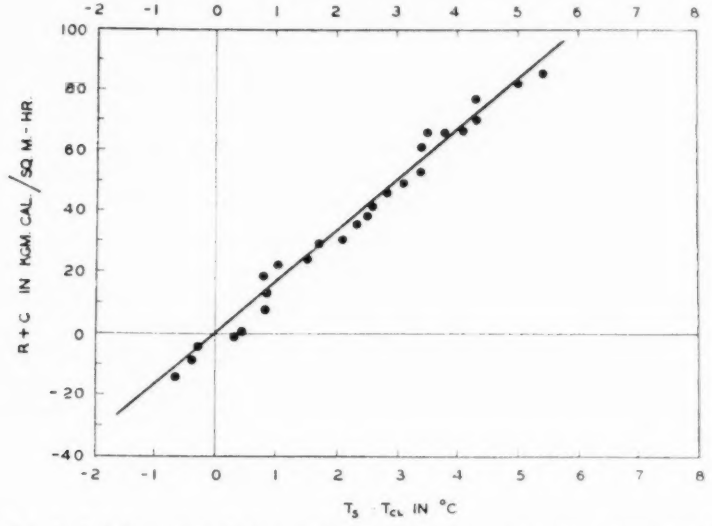



Fig. 3. Relation of radiation and convection to mean temperature gradient between skin and surface clothing.

If one takes a figure of 40 kgm.cal./m²-hr. as representative of *M-E* in the cool region, the temperature gradient from the skin to an environment of operative temperature 25.1°C., is indicated by the following:

| | | | |
|---|---|---|---|
| Skin temperature | 33.1° | Conductance of clothing | 15.8 |
| ↓ | | | |
| Clothing temperature | 30.6° | Conductance by radiation plus convection | 7.3 |
| ↓ | | | |
| Operative temperature | 25.1° | | |

*Vascular changes in the skin.* In the previous paragraph the effect on the thermal gradient to the environment of the two factors, conductance of the clothing, and conductance of the environment, was demonstrated. The conductance of the clothing and of the environment are essentially physical properties and remain invariant over the range of experimentation. Skin conductance, on the other hand, varies considerably with experimental conditions and is an important physiological property of the body system.

The physiological importance of the conductance of the skin has long been recognized by workers in the field of animal metabolism (Kleiber, 1932, 1937). For the human body its importance was most satisfactorily

demonstrated by Burton and Bazett (1936) who, for the nude subject in a water bath, correlated changes in conductance with vascular changes in the skin. Comparable results were reported by us (Winslow, Herrington, and Gagge, 1937a) for a nude subject in the copper booth. More recently, Hardy and Dubois (1937) have presented further results for their calorimeter subjects.

The fundamental physical law which describes the flow of heat from the interior of the body to the skin surface is

Total heat flow to unit area of skin ($E + R + C$ in kgm.cal./m²-hr.) = Thermal Conductance ($K$ in kgm.cal./m²-hr.-°C.) of the skin × Temperature Gradient to the skin ($T_B - T_S$ in °C.). (5)

The total heat flow includes both the heat transferred to the skin by physical conduction alone and that transported by the moving blood stream. The former may be assumed to depend on the specific conductivity ($k$) of the tissues, *per se*, on the linear depth of the thermal gradient ($d$), and on the temperature gradient ($T_B - T_S$); the latter on the specific heat of the blood ($s$), the volume of blood flow per unit time ($f$), and on the temperature drop ($T_B - T_S$) to the skin surface. In equational form this may be written

$$E + R + C = sf\,(T_B - T_S) + k/d\,(T_B - T_S),$$

or

$$= (sf + k/d)\,(T_B - T_S), \tag{6}$$

where $s$ is given in kgm.cal./liter-°C., $f$ in liter/m²-hr., $k$ in kgm.cal./m²-hr.-°C./cm., and $d$ in cm. Therefore, from (5) and (6) in terms of blood flow, $f$, and depth of gradient, $d$, the conductance, $K$, is described by the relation

$$K = sf + k/d. \tag{7}$$

Equation (7) does not describe an exact relationship between effective conductance, blood flow, and conductivity, as it assumes that the blood loses its heat wholly at the skin surface and that the specific conductivity is unchanged by the blood flow. Since the blood, in passing from the heart to the skin surface, cannot cool by more than the difference, $T_B - T_S$, the blood flow, $f$, as used in (7), represents the minimum flow effective in the transfer of heat across the temperature gradient to the skin surface. If one considers $f$ as a gross measure of the "effective" skin circulation and assumes similar interpretations for $k$ and $d$, we believe (7) to be sufficiently valid for the general arguments to follow.

Comparative changes in thermal conductance of the skin for both the clothed and the nude state are illustrated in the lower graph of figure 2 when plotted against operative temperature. It will be noted that below 33.5°$T_O$ the nude curve lies above that of the clothed, while for temperatures higher than 33.5°$T_O$, the reverse is true. In the operative tempera-

ture range where body cooling is effective, the general variation in conductance is small. Within the respective zones of vasomotor regulation, the change of conductance with operative temperature is greater for the nude than for the clothed subject. This is as one would expect, since the clothing increases the ease, as well as the possible range of vasomotor regulation. Once evaporative regulation has begun, it is interesting to note that the increase in conductance with $T_O$ is very much greater for the clothed than for the nude state. Another important observation is the fact that the

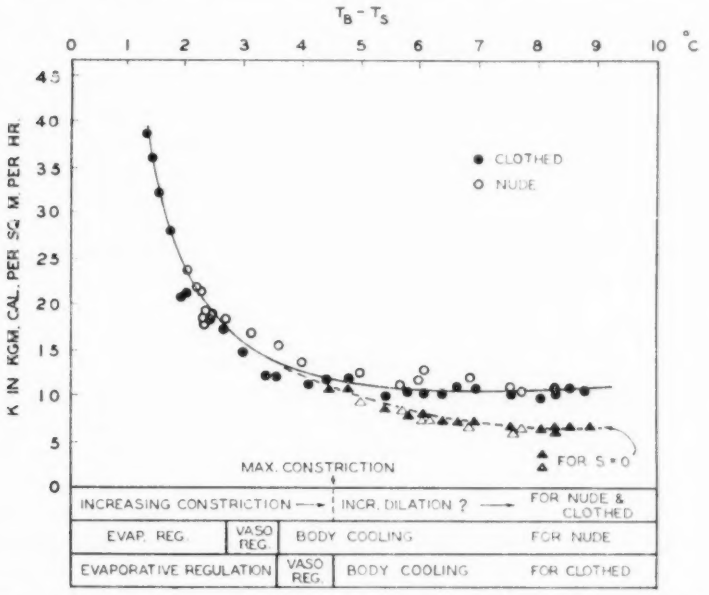



Fig. 4. Relationship between mean temperature gradient through superficial body tissues and conductance.

onset of evaporative regulation, when comparing the nude and clothed subjects, is independent of the magnitude of the conductance, $K$.

The physiological properties concerned are best demonstrated by plotting the total conductance, $K$, against the temperature gradient through the skin ($T_B - T_S$) as shown in figure 4. For any point on this curve the product of the ordinate and abscissa values equals the flow of heat to the skin surface ($M + S$ or $E + R + C$). Overlooking for the moment the slight excess in conductance for the nude over the clothed, which may represent a constitutional difference between the subjects, it is evident that both curves tend to flatten out at a temperature gradient of 4.5°C. It seems logical, therefore, to assume that at 4.5°C., vasoconstriction is a

maximum for both nude and clothed states. At 4°C. gradient, a minimum in the value of conductance was observed by Burton and Bazett (1936) for subjects with a slightly lower metabolic level than ours. Therefore, in equation (7), if $f$ is considered zero at maximum constriction, and if one uses Lefèvre's value (1911) of 0.00066 gram cal./cm²-sec.-°C./cm., or 23.8 kgm.cal./m²-hr.-°C./cm., for the specific conductivity of the tissues ($k$), the depth of gradient ($d$), is 2.2 cm., for the critical conductance, $K$, of 10.8 at 4.5°C. gradient. This value of 2.2 cm. is in general agreement with the direct measurements of Bazett and McGlone (1927).

Below 4.5°C. gradient, the depth of gradient, $d$, remains theoretically a constant physical property of the body, as long as the mean temperature of the body remains constant, and the increase in conductance, $K$, is entirely due to an increase in blood flow. From figures 2 and 4, it may be seen that clothing tends to increase greatly the peripheral circulation for a given operative temperature in the hot region. Further, this increased circulation is not necessarily associated with an increased total evaporation, although the secretion of sweat may be increased considerably under the clothing. If the specific heat of the blood is considered unity, equation (7) may be stated as

$$K = f + 23.8/d. \tag{8}$$

For the maximum observed conductance of 38.9 for the clothed subject at 1.3°C. gradient and at 39°$T_O$ (since $d$ = 2.2 cm.) the minimal increase in peripheral circulation, $f$, is 28 liters/m²-hr., which is approximately 21 per cent of the normal specific cardiac output (132 liters/m²-hr., as given by Grollman, 1934). This percentage may be correlated roughly with a 24 per cent increase in cardiac output over normal, deduced from data reported by Grollman (1930) for a clothed subject at 39° environmental temperature. For the nude at 39°$T_O$, the conductance, 23.8, represents an increase in peripheral flow of 13 liters per square meter, a value approximately 10 per cent of the normal cardiac output.

In figure 4, for temperature gradients greater than 4.5°C., it may be seen that the conductance remains relatively constant in value, whether nude or clothed, although the nude values are slightly higher. All the points in this region of the curve lie in the zone of body cooling. Physically, the important question to be answered is, whether or not there is any change in blood flow ($f$) or depth of gradient ($d$) necessary to maintain this relatively constant conductance, $K$. If $d$ should remain constant for the lower operative temperatures, then, from purely physical reasoning, one would expect the rate of change of rectal temperature to be a correct index of storage. This is well known not to be true for this zone and has been demonstrated quantitatively by one of us (Gagge, 1936). With lowering operative temperature and lowering skin temperature,

a simultaneous lowering of mean body temperature must eventuate. We are at once led to the conclusion that, since the rectal temperature does not fall as quickly as the skin temperature in the zone of body cooling, the depth of gradient, $d$, cannot remain constant but must increase toward the colder regions; further, on account of the general constancy of total conductance in these regions, this increase in $d$ must be balanced by a small increase in peripheral blood flow, $f$. By increase in peripheral blood flow, we do not mean necessarily an increased circulation through the subcutaneous capillary bed alone (vasodilatation in the classical sense), such as occurs above 4.5° gradient, but increased circulation of blood anywhere in the temperature gradient itself. Physically, this could include circulation through capillaries on the surface of the muscles, if the gradient depth, under such cold conditions, extended sufficiently deep.

In attempting to evaluate such a small increase in general peripheral circulation toward colder temperatures, it follows, when no storage occurs, that the conductance at any time must be large enough to allow dissipation of the metabolic heat across the temperature gradient. This minimal theoretical conductance is evaluated by the ratio of the metabolism to the temperature gradient itself. These limiting values, so calculated from tables 2 and 3, are plotted in figure 4 ($S = 0$), for both the nude and clothed states. Physically, if the peripheral blood flow were zero, the maximum increase in gradient depth would be defined by these minimal conductance values, and as one sees from (8) the gradient depth of 2.2 cm., at 4.5°C. gradient, increases to 3.6 cm., at 8.8°C. gradient. Therefore, to maintain the constant thermal conductance experimentally observed, the peripheral blood flow must increase from zero at 2.2 cm. gradient depth to 4.3 liters/m²/hr. at 3.6 cm. depth, i.e., an increase in gradient depth of 1.4 cm. is balanced by an increase in peripheral blood volume of roughly 3 per cent of normal cardiac output.

From tables 2 and 3 in association with figure 1, the zones of evaporative regulation, vasomotor regulation, and body cooling have been indicated in the lower part of figure 4. It is interesting to note that, when one compares the nude and clothed subjects, the limits of these zones are not related to the temperature gradient to the skin ($T_B - T_S$).

In a previous communication from this laboratory, we presented evidence showing that a considerable vascular change may occur in the zone of body cooling (Herrington, Winslow, and Gagge, 1937). For two thermally equivalent conditions, marked differences were observed, both in skin temperature and conductance, where equal air and wall temperatures were replaced by a low air temperature and a high wall temperature. If $f$ and $d$ were physiologically invariant for the cold region, this difference could not have been observed.

*Reaction to caloric demand of the clothed subject in contrast with the nude*

*subject.* In our earlier discussion we compared the physiological properties of clothed subjects with those of nude subjects only in regard to variations with operative temperature. Although operative temperature is the important variable from the practical viewpoint of describing the environment in the simplest terms, this variable does not give us an equivalent basis on which the physiological properties of both the nude and clothed

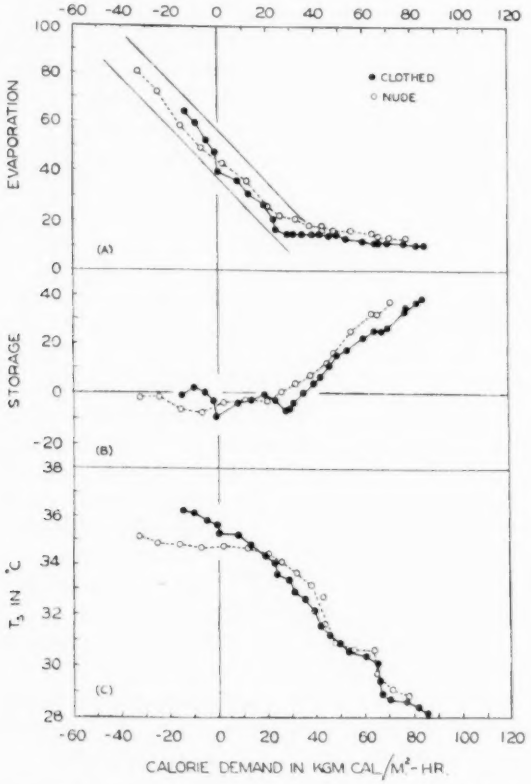



Fig. 5. Evaporation, storage, and skin temperature for clothed and nude subjects in relation to environmental calorie demand.

subjects may be compared. In one case the environment is operating on a clothed surface and in the other on the nude skin. So far, our reference of comparison has been the surface actually exposed to the wall and air temperatures. It seemed of interest to substitute, as a reference point, the skin surface for both cases and compare the clothed subjects with the nude for identical values of the *calorie demand*; i.e., the sensible-calorie

loss demanded by the thermal properties of the environment from the two respective skin surfaces. We have, therefore, re-plotted all our basic data against $R + C$.[3] The graphs for skin temperature, evaporation, and storage, are of special interest and are presented in figure 5.

In figure 5 the curves for the total evaporation loss for both nude and clothed subjects are practically identical. In the range of calorie demand from $-30$ to $+30$, the decrease in evaporation loss exactly balances the increase in demand. This is evidenced by the slope of these curves which lie symmetrically between parallel bars drawn at 45° with the horizontal, and demonstrates the remarkable thermal adaptability of the body. It is of further interest that, although the evaporation exactly balances the change in calorie demand, this compensation involves a slight excess of body temperature, as indicated by the prevalent negative storages (warming of the body) for this region. The inflection point at $+30$ demand is of additional significance; the metabolism for this region is approximately 48 while the evaporation is 18 and minimal.

Below the point of incipient body cooling, $T_s$ is determined by the internal temperature of the body, the rate of heat flow from the interior of the body to the skin, and the calorie demand of the environment. Since the internal body temperature varies over a very limited range with operative temperature, for a given calorie demand $T_s$ should be essentially the same for both clothed and nude subjects in this cold zone (calorie demand of $+25$ or more); i.e., the mean skin temperatures should be governed physically only by calorie demand. Unless the slight excess for the nudes between $+20$ and $+40$ demand be significant, this statement is generally validated in the lower curves of figure 5. As a result of the identity of $T_s$, storage is also the same for both clothed and nude subjects at a given calorie demand.

For the cold zone the regional behavior of the various areas of the body is quite different for the clothed and nude subject, as indicated in figure 6. The variation in head temperature with calorie demand for the nude and clothed subjects is almost the same. However, in the case of the other three segmental skin areas, marked differences occur. We note that both the upper and lower extremities of the clothed subject have lower skin temperatures than those of the nude subjects (for a given value of $R + C$). This difference is most marked for the upper extremities. The skin temperatures of the trunk, on the other hand, show an opposite effect; the temperature is higher for the clothed than for the nude. On physical grounds alone the effect of clothing would be to favor the outflow of heat to the extremities, increasing at the same time their temperature and also

[3] Since the evaporation takes place wholly on the skin surface of the clothed subject for the data reported here, the nude and the clothed subjects act as identical thermal systems in relation to $R + C$, the calorie loss by radiation and convection.

that of the trunk, though in less degree. The greater drop in temperature of the extremities with a simultaneous excess in trunk temperature (for the clothed as compared with the nude subjects) is an evidence of physiological

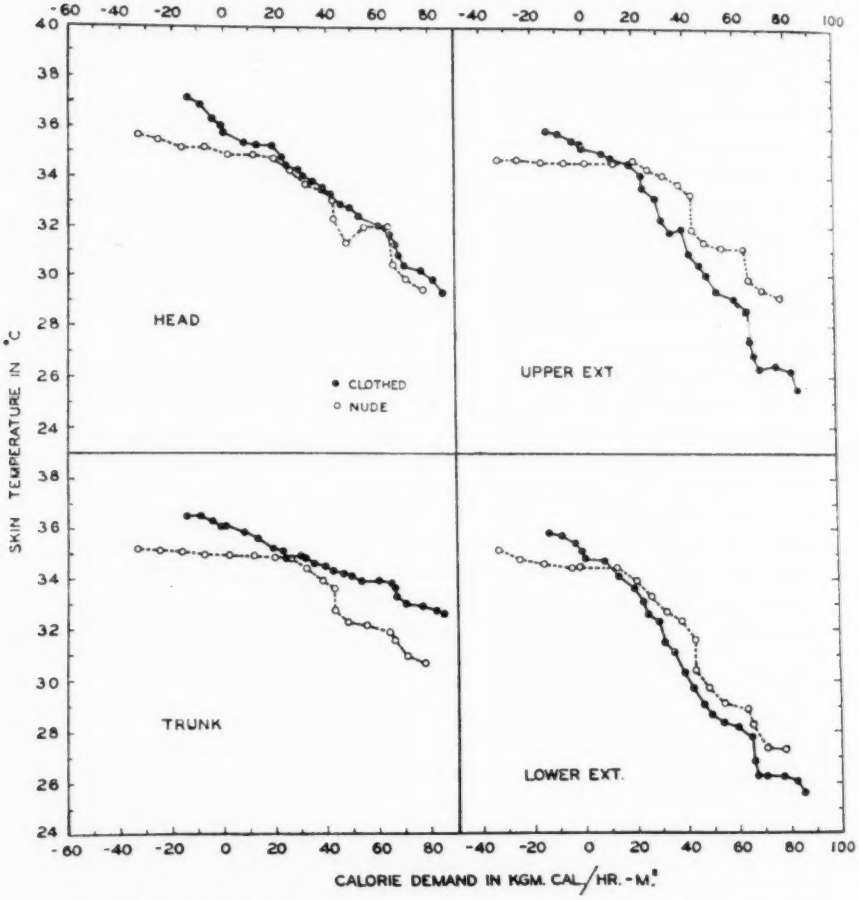



Fig. 6. Regional skin temperature of clothed and nude subjects in relation to environmental calorie demand.

adjustment. This phenomenon again demonstrates the importance of peripheral blood flow and its distribution in the zone of body cooling.

For calorie demands below 25 kgm.cal./m²-hr., where the warm zone lies, it can be seen from figure 5 and our tables, that for both the nude and clothed body, changes in metabolism, storage, and evaporation are identi-

cal in relation to calorie demand. The essential difference between the two cases lies in the response of skin temperature. For the nude subject the mean skin temperature, as well as the various segmental skin temperatures, tends to remain constant with increasing operative temperature after the onset of evaporative regulation. For the clothed subject, however, the skin temperature continues to rise slowly after the onset of evaporation. The calorie balance for both nude and clothed remains the same. As can be seen in tables 2 and 3 these higher skin temperatures of the clothed body are associated with a considerably higher conductance of the peripheral tissues, i.e., increased peripheral flow of blood. In figure 6, however, one may note that, with the exception of the trunk, the skin temperatures of the head and extremities level off at the onset of evaporation,

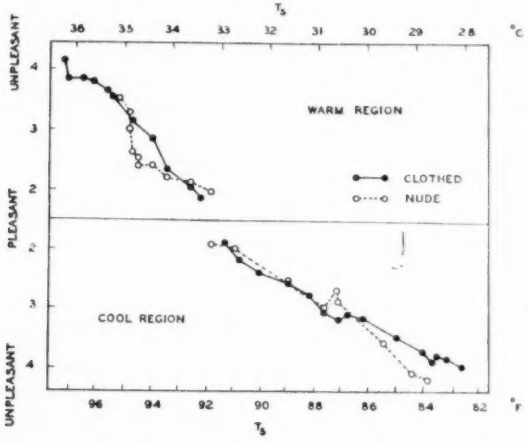



Fig. 7. Sensations of pleasantness of clothed and nude subjects in relation to skin temperature.

and do not rise again until at zero calorie demand, when the body begins to receive heat from the environment. At this point the clothing temperature becomes greater than the skin temperature, and the increased vascular response of the body tends to reduce the transfer of heat from the clothing surface to the skin, forming essentially a protective mechanism against the inflow of heat to the body.

*Sensations of comfort.* As in previous studies (Winslow, Herrington, and Gagge, 1937a), our subjects, at the close of each experiment, were asked to express their reactions to the environmental situation on a scale of five points as follows: 1, very pleasant; 2, pleasant; 3, indifferent; 4, unpleasant; 5, very unpleasant. Although the unfavorable comfort votes were expressed merely as "unpleasant" or "very unpleasant," it is

obvious, from inspection of tables 2 and 3, that there is an optimum value at an operative temperature of 26.5°–30° and a steady increase in vote values on each side of this optimum. On one side "unpleasant" means "too warm," and on the other side "too cool." In preparing figure 7, therefore, we have plotted the votes in opposite directions on either side of the votes of 1.5 or less.

We have been interested in finding some particular physiological variable with which the sensation of pleasantness could be most closely correlated. In an earlier paper (Winslow, Herrington, and Gagge, 1937b) we concluded that for the nude subject, sensations of pleasantness could be best correlated with skin temperature in the region of body cooling and with wetted area in the zone of evaporative regulation.

The relationship to skin temperature is even more striking with the clothed subjects and appears in both the hot and cold zone, as indicated in figure 7. There is also a correlation of comfort with wetted area on the hot side, but it is quite a different relation for the clothed and nude subjects. Conditions associated with total evaporation of 30 to 65 kgm.cal./m²-hr. showed votes of 3.5–3.8 for the clothed, and 2.4–2.6 for the nude subjects. On the other hand, a given skin temperature was associated with the same comfort vote for both clothed and nude subjects. We may, therefore, conclude that skin temperature is the primary factor controlling sensations of thermal pleasantness, in both the hot and cold zone.

## SUMMARY

The partition of heat loss from a normally clothed subject in a semi-reclining position shows that he is able to tolerate a difference of 8°C. between skin and environmental temperature before body cooling sets in at 25°$T_o$ (as compared with 29°$T_o$ for the nude subject). Between 25° and 29°$T_o$, thermal equilibrium of the clothed body is maintained by vasomotor regulation. Above 29°, evaporative regulation sets in.

For equal wall and air temperatures and a turbulent air movement of 5 meters per minute, 55 per cent of the total loss by radiation plus convection is lost in radiation; the transfer of heat from the clothing surface to the environment by radiation and convection is expressed by the environmental constant, 7.34 kgm.cal./m²-hr.-°C. The physical conductance of the clothing is 15.8 kgm.cal./m²-hr.-°C.

The thermal conductance of the skin has been expressed quantitatively in terms of an effective peripheral blood flow and a mean depth of the thermal gradient. Subsequent analysis shows that, in the zones of vasomotor regulation and evaporative regulation, blood flow is the principal variable in conductance. In the zone of body cooling (below 25°$T_o$), it is shown that with increasing depth of thermal gradient, as indicated by

a general increased rate of cooling of the body, the peripheral flow of blood may slowly increase.

A range of 4°–4.5°C. difference between internal tissues and skin represents a point of minimal conductance and presumable maximum vasoconstriction.

In the range of temperatures and humidities studied, it has been shown that heat loss by evaporation takes place at the skin surface and not in the folds of the clothing. Since this is the case, the calorie demand of the environment, as expressed in total loss by radiation plus convection, acts ultimately on the skin whether clothed or unclothed. By plotting various physiological factors against calorie demand, it has been shown that the gross physiological responses of the clothed body when compared with the nude are broadly identical. In detail the following differences are observed:

1. In the zone of body cooling, for calorie demands greater than 30 kgm.cal./m²-hr., the responses of mean skin temperature for both nude and clothed subjects are identical for a given calorie demand. However, segmental values composing these means vary considerably. The head temperatures, in general, are the same; but for the upper and lower extremities, the skin temperatures of the clothed are lower than those of the nude, while for the trunk, the opposite is true.

2. In the zone of evaporative regulation, for calorie demands of less than 25 kgm.cal./m²-hr., it is shown (for both nude and clothed subjects) that the loss by evaporation increases in calories by the same amount that the demand decreases. In this zone the essential difference is a markedly greater rise in skin temperature and thermal conductance for the clothed subjects.

Throughout the range of experiments, sensations of pleasantness closely follow skin temperature and show the same relation to skin temperature for both clothed and nude subjects.

## REFERENCES

ALDRICH, L. B. Study of body radiation. Smithsonian Miscellaneous Collections, Publication no. 2980, 1928.

BAZETT, H. C. AND B. MCGLONE. This Journal **82:** 415, 1927.

BURTON, A. C. AND H. C. BAZETT. This Journal **117:** 36, 1936.

DUBOIS, D. AND E. F. DUBOIS. Arch. Int. Med. **15:** 868, 1916.

GAGGE, A. P. This Journal **116:** 656, 1936.

GAGGE, A. P., L. P. HERRINGTON AND C.-E. A. WINSLOW. Am. J. Hyg. **26:** 84, 1937.

GROLLMAN, A. This Journal **95:** 263, 1930.
The cardiac output of man in health and disease. Charles Thomas, p. 87, 1932.

HARDY, J. D. J. Clin. Investigation **13:** 593, 1934.

HARDY, J. D. AND E. F. DUBOIS. Proc. Nat. Acad. Sci. **23:** 624, 1937.

HARDY, J. D. AND E. F. DUBOIS. J. Nutrition **15:** 477, 1938.
HERRINGTON, L. P., C.-E. A. WINSLOW AND A. P. GAGGE. This Journal **120:** 133, 1937.
KLEIBER, M. Hilgardia **6:** 315, 1932.
Ann. Rev. Biochem. **375**, 1937.
LEFÈVRE, I. Chaleur Animale. Sect. III, p. 398, 1911.
WINSLOW, C.-E. A., L. P. HERRINGTON AND A. P. GAGGE. This Journal **116:** 641, 1936a.
This Journal **116:** 669, 1936b.
This Journal **120:** 1, 1937a.
Am. J. Hyg. **26:** 103, 1937b.

# THE RELATIVE INFLUENCE OF RADIATION AND CONVECTION UPON THE TEMPERATURE REGULATION OF THE CLOTHED BODY

C.-E. A. WINSLOW, L. P. HERRINGTON, AND A. P. GAGGE

*Contribution no. 23, from the John B. Pierce Laboratory of Hygiene, New Haven, Connecticut*

Received for publication June 11, 1938

In a previous communication from this laboratory (Herrington, Winslow, and Gagge, 1937), we have shown that nude subjects exhibit markedly different reactions to heat interchanges with the environment as influenced respectively by convection and radiation. It was apparent in these studies that the unclothed body shows a greater fall in skin temperature with cold air and hot walls than with the same operative temperature (identical thermal demand) produced by air and walls whose temperatures are alike.

During the past year, we have completed a series of experiments with clothed subjects which have been reported in their more general aspects in a preceding communication (Gagge, Winslow, and Herrington, 1938). In the paper cited, our results were analyzed from the standpoint of operative temperature, without reference to difference between air and walls. It is the purpose of the present communication to consider the data from the standpoint of the differential influence of radiation and convection.

The general conditions of the experiments are described in the paper cited above. That paper, however, contained only moving averages of mean values for two subjects for given operative temperatures, without the component air and wall temperatures. The full data on which the present communication is based are presented in tables 1 and 2 for two subjects (VII and IX). Each horizontal line in the tables represents the mean of six experiments made with the same combination of environmental conditions. The groups of experiments have been arranged under three headings as follows:

Low radiation. Air and walls not differing by over 2°C. (in 11 of the 14 series the difference was less than 0.5°C.).

Medium radiation. Radiant temperature of walls 6.5°–15.5°C. above air temperature.

High radiation. Radiant temperature of walls 17°–34.5°C. above air temperature.

TABLE 1*

*Subject VII—Clothed*

| EXPERIMENT | $T_O$ | $T_A$ | $T_W$ | $T_H$ | $T_U$ | $T_T$ | $T_L$ | MEAN $T_S$ | MEAN $T_{Cl}$ | $T_B$ | $M$ | $S$ | $E$ | $R$ | $C$ | $WA$ | K | COMFORT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | °C. | °C. | °C. | °C. | °C. | °C. | °C. | °C. | °C. | °C. | kgm. cal./ m²-hr. | kgm. cal./ m²-hr. | kgm. cal./ m²-hr. | kgm. cal./ m²-hr. | kgm. cal./ m²-hr. | per cent | kgm. cal. m²-hr.-°C. | |
| **Low radiation** | | | | | | | | | | | | | | | | | | |
| 21 | 37.1 | 37.1 | 37.1 | 37.2 | 36.4 | 36.3 | 36.3 | 36.4 | 37.9 | 37.6 | 51 | 17 | −59 | −6 | −3 | 72 | 58.4 | 4.3 |
| 13 | 32.1 | 32.1 | 32.1 | 35.8 | 35.6 | 36.2 | 35.6 | 35.3 | 35.2 | 37.6 | 49 | 13 | −35 | −16 | −11 | 39 | 27.2 | 4.0 |
| 9 | 27.1 | 26.2 | 27.8 | 34.3 | 34.5 | 35.2 | 33.8 | 34.4 | 32.9 | 37.2 | 47 | 17 | −14 | −26 | −24 | 15 | 23.1 | 1.7 |
| 1 | 22.0 | 22.0 | 22.0 | 33.6 | 32.8 | 34.3 | 30.7 | 32.6 | 30.5 | 37.1 | 47 | 32 | −11 | −37 | −30 | 14 | 17.3 | 2.0 |
| 5 | 16.3 | 15.3 | 17.2 | 32.1 | 28.2 | 33.9 | 27.2 | 29.8 | 26.9 | 37.3 | 45 | 51 | −12 | −43 | −41 | 14 | 12.3 | 3.0 |
| 17 | 10.6 | 10.4 | 10.6 | 29.9 | 26.9 | 33.3 | 25.4 | 28.5 | 24.0 | 37.3 | 45 | 66 | −9 | −54 | −48 | 13 | 12.6 | 3.7 |
| 25 | 5.4 | 5.4 | 5.4 | 26.3 | 24.2 | 32.3 | 22.2 | 26.1 | 20.9 | 37.1 | 52 | 74 | −10 | −61 | −55 | 17 | 11.0 | 4.0 |
| **Medium radiation** | | | | | | | | | | | | | | | | | | |
| 22 | 38.9 | 32.7 | 43.7 | 36.6 | 35.4 | 36.4 | 36.1 | 36.1 | 36.6 | 37.4 | 49 | −7 | −65 | 37 | −14 | 70 | 30.3 | 3.1 |
| 14 | 33.6 | 27.0 | 38.5 | 35.8 | 35.2 | 35.8 | 34.7 | 35.2 | 34.8 | 37.5 | 47 | 0 | −39 | 19 | −27 | 44 | 20.7 | 3.3 |
| 10 | 28.4 | 21.5 | 33.6 | 34.8 | 33.4 | 34.8 | 32.5 | 33.6 | 32.3 | 36.8 | 47 | 0 | −14 | 5 | −38 | 15 | 14.4 | 1.8 |
| 2 | 22.3 | 15.7 | 27.3 | 33.7 | 30.7 | 33.8 | 28.2 | 30.8 | 28.4 | 36.8 | 45 | 14 | −19 | 5 | −45 | 26 | 9.8 | 3.0 |
| 6 | 16.4 | 9.7 | 21.4 | 32.2 | 26.6 | 33.7 | 25.5 | 28.7 | 24.7 | 37.2 | 47 | 33 | −14 | −13 | −53 | 14 | 9.3 | 3.6 |
| 18 | 12.7 | 4.7 | 18.7 | 27.8 | 23.9 | 32.1 | 22.2 | 26.0 | 21.4 | 36.9 | 52 | 25 | −7 | −11 | −59 | 12 | 7.0 | 4.2 |
| **High radiation** | | | | | | | | | | | | | | | | | | |
| 23 | 40.4 | 27.7 | 50.0 | 37.1 | 35.7 | 36.7 | 36.3 | 36.3 | 36.9 | 36.9 | 47 | −19 | −63 | 67 | −32 | 55 | 24.0 | 4.0 |
| 24 | 39.9 | 21.7 | 53.7 | 37.2 | 35.8 | 36.9 | 35.9 | 36.3 | 36.2 | 37.4 | 48 | −21 | −64 | 88 | −51 | 62 | 23.2 | 4.0 |
| 16 | 35.2 | 15.6 | 50.1 | 36.2 | 34.6 | 36.9 | 34.9 | 35.6 | 33.5 | 37.3 | 45 | −35 | −34 | 87 | −63 | 32 | 5.8 | 3.5 |
| 15 | 34.3 | 21.6 | 43.9 | 34.4 | 34.6 | 35.8 | 34.9 | 35.1 | 33.9 | 37.2 | 48 | −13 | −43 | 51 | −43 | 42 | 17.1 | 3.7 |
| 11 | 30.4 | 16.9 | 40.6 | 33.1 | 33.3 | 35.8 | 32.8 | 33.8 | 31.4 | 37.2 | 43 | −21 | −18 | 47 | −51 | 18 | 6.3 | 2.0 |
| 12 | 29.8 | 11.4 | 43.6 | 35.5 | 33.8 | 35.7 | 32.7 | 34.1 | 31.4 | 37.2 | 46 | −17 | −20 | 61 | −70 | 19 | 14.1 | 2.0 |
| 4 | 23.9 | 5.4 | 37.9 | 33.6 | 29.8 | 34.5 | 27.2 | 30.4 | 28.1 | 37.1 | 45 | 1 | −14 | 48 | −80 | 17 | 6.8 | 2.0 |
| 3 | 22.5 | 10.0 | 31.9 | 34.5 | 29.2 | 34.3 | 27.2 | 30.3 | 27.3 | 37.2 | 45 | 9 | −16 | 23 | −61 | 20 | 8.3 | 3.4 |
| 7 | 17.8 | 5.4 | 27.1 | 32.1 | 25.9 | 33.6 | 25.3 | 28.5 | 24.7 | 37.3 | 48 | 22 | −12 | 10 | −68 | 12 | 7.9 | 3.7 |
| 19 | 12.2 | 0.1 | 21.3 | 28.7 | 24.3 | 31.5 | 25.0 | 27.1 | 21.1 | 37.1 | 51 | 32 | −10 | 1 | −74 | 15 | 8.3 | 5.0 |

* In tables 1 and 2 the basic observed data are: $T_O$, the operative temperature in °C.; $T_H$, the mean skin temperature of head in °C.; $T_U$, the mean skin temperature of upper extremities in °C.; $T_T$, the mean skin temperature of trunk in °C.; $T_L$, the mean skin temperature of lower extremities in °C.; $T_S$, the mean skin temperature found by averaging $T_H$, $T_U$, $T_T$, and $T_L$ weighted by the factors 7, 21, 31, and 41, respectively; $T_B$, the rectal temperature in °C.; $T_{Cl}$, the mean temperature of the exposed clothing and skin surfaces over the entire body in °C., weighted as in the case of $T_S$; $M$, the metabolism expressed in kgm. cal./m²-hr.; $E$, the total evaporation loss, expressed in kgm.cal./m²-hr. Comfort vote, expressed numerically by 1 (very pleasant), 2 (pleasant), 3 (indifferent), 4 (unpleasant), 5 (very unpleasant).

The derived data are: $R$, radiation exchange in kgm.cal./m²-hr. between mean exposed skin-clothing surface and environment. When positive, $R$ denotes a gain in heat from the environment; when negative, a loss. $C$, convection loss in kgm.cal./m²-hr. from the exposed skin-clothing surface to the ambient air. $S$, storage, or rate of body cooling when positive; body heating, when negative; the value of $S$ is so chosen that $M + S$ equals the net heat exchange between $E + R + C$. $WA$, wetted area expressed as a percentage of maximum wettedness of the moist surfaces of the body, found by dividing the rate of evaporation, $E$, by the product of the difference between the saturation vapor pressure at skin temperature and the vapor pressure of the ambient air in centimeters of Hg, with a factor 27.9 kgm.cal./m²-hr.-cm. of Hg. $K$, the conductance of the peripheral tissues, as defined by the relation $E + R + C/T_B - T_S$, and expressed in kgm.cal./m²-hr.-°C.

For low radiation conditions, heat losses due to radiation and convection were nearly equal (when wall and air were exactly equal, 56 per cent radiation and 44 per cent convection). For high radiation conditions,

TABLE 2*

*Subject IX—Clothed*

| EXPERIMENT | $T_O$ | $T_A$ | $T_W$ | $T_H$ | $T_U$ | $T_T$ | $T_L$ | MEAN $T_S$ | MEAN $T_{Cl}$ | $T_B$ | $M$ | $S$ | $E$ | $R$ | $C$ | $W.A$ | K | COMFORT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | °C. | °C. | °C. | °C. | °C. | °C. | °C. | °C. | °C. | °C. | kgm. cal./ m²-hr. | kgm. cal / m²-hr. | kgm. cal./ m²-hr. | kgm. cal./ m²-hr. | kgm. cal./ m²-hr. | per cent | kgm. cal. m²-hr.-°C. | |
| **Low radiation** | | | | | | | | | | | | | | | | | | |
| 21 | 37.9 | 37.9 | 37.9 | 37.9 | 36.7 | 36.7 | 36.6 | 36.7 | 38.3 | 37.7 | 54 | 14 | −65 | −2 | −1 | 82 | 72.0 | 4.7 |
| 13 | 31.9 | 31.9 | 31.9 | 35.7 | 35.4 | 35.7 | 35.3 | 35.5 | 34.9 | 37.1 | 49 | 11 | −41 | −10 | −9 | 49 | 38.5 | 4.3 |
| 9 | 26.1 | 25.8 | 26.4 | 34.4 | 34.5 | 35.2 | 34.3 | 34.6 | 32.9 | 37.0 | 53 | 4 | −12 | −24 | −21 | 14 | 24.0 | 1.3 |
| 1 | 22.3 | 22.3 | 22.3 | 33.1 | 32.8 | 34.6 | 32.4 | 33.2 | 30.7 | 37.0 | 50 | 17 | −13 | −29 | −25 | 16 | 17.8 | 2.2 |
| 5 | 15.1 | 15.1 | 15.1 | 31.5 | 29.1 | 33.7 | 29.3 | 30.7 | 27.3 | 36.7 | 56 | 20 | −9 | −41 | −36 | 12 | 12.6 | 2.0 |
| 17 | 9.9 | 9.9 | 9.9 | 30.2 | 27.4 | 32.6 | 27.5 | 29.2 | 24.2 | 36.4 | 58 | 39 | −9 | −45 | −43 | 13 | 13.5 | 3.5 |
| 25 | 5.4 | 5.3 | 5.4 | 28.8 | 26.1 | 32.5 | 27.0 | 28.6 | 22.3 | 36.4 | 67 | 45 | −9 | −53 | −50 | 13 | 14.4 | 4.3 |
| **Medium radiation** | | | | | | | | | | | | | | | | | | |
| 22 | 38.8 | 32.9 | 43.7 | 37.3 | 36.1 | 36.2 | 35.5 | 35.9 | 37.4 | 37.2 | 48 | 15 | −74 | 24 | −13 | 79 | 49.3 | 4.0 |
| 38 | 36.3 | 27.2 | 43.8 | 36.5 | 35.6 | 35.7 | 34.6 | 35.3 | 35.9 | 37.1 | 48 | 1 | −53 | 30 | −26 | 58 | 27.6 | 4.0 |
| 39 | 35.2 | 31.4 | 38.2 | 36.6 | 35.8 | 35.7 | 34.9 | 35.3 | 36.3 | 37.1 | 52 | 10 | −54 | 6 | −14 | 58 | 34.9 | 4.0 |
| 14 | 34.2 | 27.8 | 39.4 | 35.6 | 35.3 | 36.2 | 34.8 | 35.4 | 35.1 | 37.2 | 48 | 2 | −41 | 16 | −21 | 43 | 25.0 | 3.0 |
| 41 | 31.0 | 27.5 | 33.9 | 35.6 | 35.1 | 35.3 | 34.0 | 34.2 | 34.3 | 37.1 | 45 | 8 | −31 | −2 | −20 | 36 | 18.7 | 3.0 |
| 42 | 30.1 | 21.4 | 37.1 | 35.7 | 34.2 | 33.6 | 32.3 | 33.3 | 33.9 | 36.7 | 46 | 4 | −23 | 10 | −37 | 26 | 15.0 | 2.2 |
| 10 | 28.3 | 21.1 | 34.2 | 34.2 | 33.8 | 34.7 | 33.3 | 33.9 | 32.9 | 36.8 | 50 | −7 | −12 | 4 | −35 | 13 | 15.5 | 1 8 |
| 2 | 22.6 | 15.8 | 28.2 | 32.6 | 30.6 | 34.1 | 29.7 | 31.4 | 28.4 | 36.7 | 52 | 3 | −16 | −2 | −37 | 20 | 10.5 | 3.7 |
| 6 | 16.5 | 9.7 | 22.1 | 30.9 | 27.3 | 33.3 | 27.7 | 29.6 | 25.5 | 36.7 | 53 | 16 | −11 | −11 | −47 | 15 | 9.6 | 4.0 |
| 18 | 12.5 | 4.4 | 19.1 | 30.3 | 25.7 | 32.9 | 27.3 | 28.9 | 22.1 | 36.6 | 66 | 6 | −10 | −9 | −53 | 13 | 9.4 | 4.0 |
| **High radiation** | | | | | | | | | | | | | | | | | | |
| 23 | 39.6 | 26.9 | 49.9 | 37.1 | 35.8 | 36.2 | 34.8 | 35.6 | 37.3 | 37.3 | 47 | −2 | −64 | 50 | −31 | 70 | 26.1 | 5.0 |
| 24 | 39.0 | 21.8 | 53.1 | 36.8 | 35.3 | 36.4 | 35.2 | 35.7 | 36.4 | 37.4 | 55 | −10 | −70 | 69 | −44 | 66 | 26.1 | 4.0 |
| 37 | 36.6 | 21.7 | 48.7 | 36.3 | 35.9 | 36.6 | 35.3 | 35.9 | 35.3 | 37.3 | 52 | −14 | −51 | 54 | −41 | 51 | 45.7 | 3.0 |
| 15 | 34.6 | 22.2 | 44.7 | 35.1 | 34.9 | 35.8 | 34.6 | 35.1 | 34.4 | 36.8 | 51 | −9 | −37 | 42 | −37 | 36 | 23.6 | 4.2 |
| 16 | 33.3 | 15.9 | 47.4 | 35.9 | 35.1 | 35.6 | 33.4 | 34.6 | 34.6 | 37.1 | 49 | −21 | −24 | 51 | −55 | 24 | 11.2 | 2.8 |
| 40 | 31.1 | 15.7 | 43.6 | 35.6 | 34.4 | 34.3 | 32.3 | 33.6 | 33.6 | 36.7 | 46 | −11 | −21 | 39 | −53 | 22 | 11.5 | 2.3 |
| 12 | 28.7 | 10.7 | 43.3 | 34.3 | 31.7 | 34.3 | 30.7 | 32.3 | 30.5 | 36.6 | 46 | −24 | −12 | 49 | −59 | 14 | 5.0 | 2.0 |
| 11 | 27.6 | 15.4 | 37.6 | 33.7 | 32.7 | 34.4 | 32.2 | 33.1 | 31.4 | 36.7 | 55 | −15 | −16 | 23 | −47 | 17 | 11.4 | 2.0 |
| 4 | 23.4 | 5.3 | 38.2 | 32.2 | 29.7 | 34.2 | 30.2 | 31.5 | 28.6 | 36.8 | 59 | −10 | −15 | 35 | −69 | 17 | 9.2 | 2.0 |
| 3 | 22.4 | 9.9 | 32.7 | 32.2 | 29.8 | 33.9 | 28.7 | 30.8 | 27.7 | 36.7 | 49 | −3 | −11 | 18 | −53 | 13 | 7.7 | 2.9 |
| 7 | 17.2 | 5.3 | 26.9 | 30.9 | 28.1 | 33.8 | 27.6 | 29.8 | 24.8 | 36.5 | 58 | 3 | −10 | 8 | −58 | 14 | 9.2 | 4.0 |
| 19 | 12.6 | 0.8 | 22.3 | 30.2 | 25.8 | 32.8 | 26.0 | 28.4 | 22.6 | 36.4 | 65 | 16 | −15 | 1 | −67 | 19 | 10.1 | 5.0 |

* See footnotes of table 1.

wall temperature was generally above skin temperature so that the body was gaining heat by radiation and losing it at a very rapid rate by convection. In one instance, on the hot side, there was a gain of 88 kilogram-

calories by radiation, and a loss of 51 kilogram-calories by convection. In a series of experiments on the cold side, 74 kilogram-calories of heat were lost by convection, per square meter of body surface per hour, with practically no radiation exchange.

*Skin temperature and storage.* In figure 1 we have plotted the mean skin temperatures against operative temperature for our two subjects and for the three conditions of low, medium and high radiation. The difference between low and high radiation effects upon skin temperature is essentially the same as that previously reported for nude subjects (Herrington, Winslow, and Gagge, 1937). With high radiation (hot walls and cold air) the skin temperature under cold conditions is 1°–3° lower than with low radiation (walls and air nearly alike) at the same operative

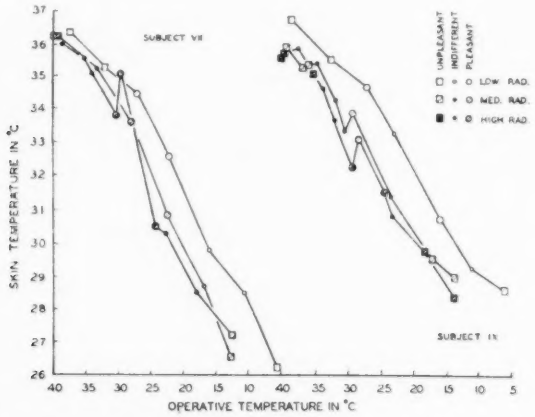



Fig. 1. The influence of radiation and convection on skin temperature at various operative temperatures.

temperatures. It appears that air temperature is far more potent than wall temperature in influencing skin temperature. Medium and high radiation are much alike in their effects, as might be expected from the fact that, even under what we call "medium radiation" conditions, air was much colder than walls. The higher skin temperature for the low radiation is clearly indicated for both subjects and at all points on the graph.

In figure 1 we have indicated by squares those points at which the subject voted that the condition was "unpleasant," and by large circles, the points at which he voted it "pleasant." Other points were reported as "indifferent." It will be noted that sensations were generally pleasant

when skin temperatures were between 30° and 35°C.; generally unpleasant when skin temperatures were higher or lower.

| *Conditions* | *Above 35° C.* | *30-35°C.* | *Below 35°C.* |
|---|---|---|---|
| Pleasant | 0 | 12 | 0 |
| Indifferent | 6 | 9 | 5 |
| Unpleasant | 12 | 0 | 8 |

Subject IX was somewhat more critical than subject VII at both ends of the scale.

We have shown (Herrington, Winslow, and Gagge, 1937) that for the nude body it is possible (in the zone of low operative temperatures) to derive a prediction equation for computing skin temperature from air temperature, wall temperature, evaporative heat loss and metabolism. From the data of the present paper we have computed a similar equation for the clothed body—again only valid for the zone of low operative temperatures where evaporation is minimal. The equation is as follows:

$$T_S = 0.29\ T_A + 0.14\ T_W + 0.11\ M + 0.08\ E + 19.52,^{1} \text{ for °C. and kg.cal./m}^2\text{/hr.}$$

The multiple correlation between $T_S$ and these four variables is 0.91 ± *S. D.* 0.03. The results are very close to those obtained for the nude subjects.

The higher coefficient for $T_A$ than for $T_W$ indicates the greater influence of air temperature than of wall temperature.

The higher skin temperature maintained when air and walls are nearly alike is, of course, manifest in a greater convection loss at a given operative temperature on the cold side and a lower convection gain on the hot side; hence, negative storage (heating of the body tissues) is less in the hot zone and positive storage (cooling of the body tissues) is greater in the cold zone with equal air and wall, than with cold air and warm walls.

The data in figure 1 refer to weighted mean skin temperatures for the entire body. Analyses of means for the various regions of the body (see tables 1 and 2) prove of considerable interest.

Means for the skin temperature of the upper and lower extremities show exactly the same picture as the means for the body as a whole, although with even greater differences between high and low radiation conditions. Graphs of head temperature show less difference between high and low radiation. Trunk temperatures are markedly lower for high and medium radiation than for low radiation, at operative temperatures between 26.5° and 32°C.

*Conductance.* The behavior of the vaso-motor regulation mechanism is

[1] When this equation is used for prediction, the calorimetric convention with reference to sign as given for $M$ and $E$ in the tables must be employed.

more clearly revealed by conductance, a factor which measures the vasomotor changes in the skin, caused chiefly (in a given individual) by the flow of blood through the superficial areas and by variations in the depth of the thermal gradient (Gagge, Winslow, and Herrington, 1938).

Values for this variable are plotted with the usual conventions in figure 2. It will be noted that values for medium and high radiation are essentially alike for subject IX, with those for medium radiation somewhat higher for subject VII. The conductance for low radiation, on the other hand, is very much higher than for either medium or high radiation, particularly in the warm and hot regions. Clearly, the influence of cold air combined with high radiation is to decrease conductance as it depresses skin temperature.

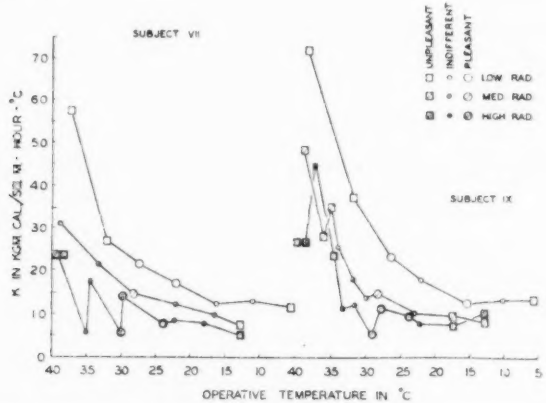



Fig. 2. The influence of radiation and convection on conductance at various operative temperatures.

It may also be noted from this graph that sensations of pleasantness are not at all correlated with conductance, the same conductance value showing the widest range of comfort votes.

*Evaporation and sweat secretion.* Analysis of the data for evaporative heat loss shows essentially identical results for both high and low radiation conditions. With high radiation, $T_s$ is slightly lower but air temperature is, of course, very much lower; therefore, the evaporative power of the air is greater. To maintain equal evaporation, the rate of sweat secretion per unit time must also be lower. This phenomenon is indicated in a graph for wetted area (fig. 3). This variable was suggested by Gagge (1937) as a measure of the extent of moisture present on the surface of the body at a given time. Computations of this variable have been made as in the contribution cited, except that since evaporation occurs on the skin

surface under the clothing, air velocity is taken as zero. It will be noted in figure 3 that high radiation conditions show a lower wetted area than low radiation conditions at values of $T_o$ above 29.5°C.

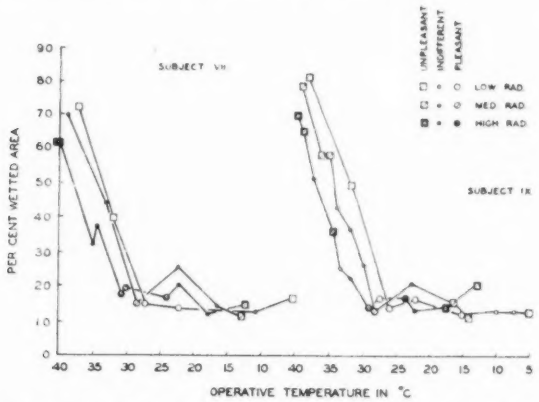



Fig. 3. The influence of radiation and convection on per cent of wetted area at various operative temperatures.

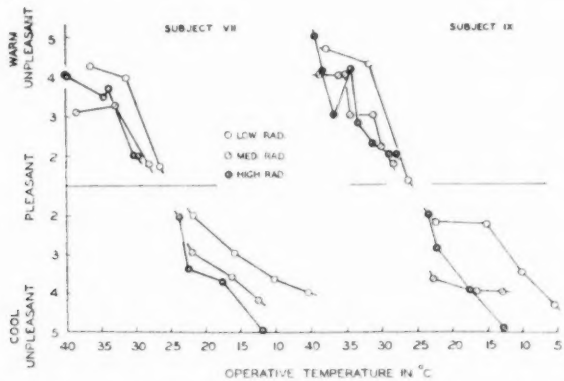



Fig. 4. The influence of radiation and convection on sensations of pleasantness at various operative temperatures.

*Sensations of pleasantness.* In all experiments the subjects indicated their reactions to the environment by votes as to their sensations of pleasantness (Winslow, Herrington, and Gagge, 1937b). A vote of "1" means very pleasant; "2", pleasant; "3", indifferent; "4", unpleasant; and "5", very unpleasant. Although no specific statement was made as to whether unpleasantness was due to heat or cold, it is reasonable to assume that at

the high operative temperatures discomfort was due to heat, at low operative temperatures to cold. On this assumption, such votes are plotted in figure 4, either above or below the median range of pleasantness.

It will be noted that comfort votes, like skin temperature, lie at a higher level for low radiation conditions than for medium or high radiation conditions. This means, of course, that the subjects were more uncomfortable in the hot zone and less uncomfortable in the cold zone with air and walls of the same temperature than with hot walls and cooler air.

With low radiation, the ideal region (votes of 2.5 or less) lies at operative temperatures between 14° and 28°C. for subject IX, and between 18° and 28°C. for subject VII.

With high radiation, votes of 2.5 or less occur at operative temperatures between 24° and 31°C. for both subjects. The upper level of operative temperature for comfort is increased about 3° by cold air combined with high radiation.

It will be noted in figure 1 that in the extreme hot zone discomfort is associated with a combination of high skin temperature and high operative temperature. With skin temperatures between 30.6° and 34.7°C., no unpleasant votes appear and very few indifferent ones. For the two subjects, there are 18 groups of experiments in this range and 11 were voted pleasant. All votes were again "unpleasant" at skin temperatures below 28.3°C. for subject VII, and below 30.5°C. for subject IX.

From figure 3, it appears that all experiments with votes of "pleasant" have wetted areas under 20—although, of course, wetted areas below 20 at low operative temperatures may, for other reasons, be associated with discomfort. With wetted areas above 40, 9 series were voted "unpleasant" and but 3 indifferent.

*Possible reasons for the differential effect of air and walls.* The phenomena here described open up tempting opportunities for speculation as to the causative factors involved. The observed results might, in part, be attributed to the fact that more heat will, obviously, be given off from the lungs in the hot-wall cold-air situation than with air and walls of equal temperature. Quantitatively, however, our computations indicate that such an explanation could not account for more than a small fraction of the observed differences. The evaporative heat loss must also be slightly greater with the cold-air and hot-walls on account of the greater moisture demand; though, again, any such difference is too slight to appear in our graphs. With the cold air, too, there must be some tendency for increased convection currents about the body; but here, also, the influence is negligible (Winslow, Herrington and Gagge, 1936).

We are, on the whole, more inclined to attribute the observed differences to local rather than general stimuli of the cold air. Hardy and Oppel (1938) have recently demonstrated interesting differences in the response

of hot and cold end organs, and find that "cold radiation produces about twice the change in skin temperature, calorie for calorie, as does hot radiation"; and this more marked physiological response to cold than to heat is a matter of general knowledge. If cold air and hot radiation could produce separate and independent effects, the observed phenomena would be easily explained. We cannot see how this can be the case, since the type of radiant energy used in our experiments is 99.9 per cent absorbed by a layer of skin 1 mm. in thickness (Hardy and Muschenheim, 1934) and, therefore, one would expect the two influences of radiation and convection to be merged in a single operative temperature before either can affect the sensory end-organs. It is, however, very probable that local or temporary stimuli of the cold air may produce the observed results. This might happen in at least three ways: by local chilling of the membranes of the upper respiratory tract (which might be far more effective in stimulating vaso-constriction than would be expected from its direct effect on calorie loss); by local chilling of the body by the metal supports of the chair in which the subject reclines (although the seat of the chair is cloth); and by temporary increase in convection loss when the subject enters the room or moves his arms or body (since this increase would be influenced only by air and not by wall temperatures).

To study the influence of such local chilling we carried out the experiments illustrated in figure 5.

Our two clothed subjects were observed in a room at 20°C. in seated position in ordinary straight-backed chairs. For the first half-hour, they were wrapped in blankets to secure a standard condition of vaso-dilatation. Then the blankets were removed and the subjects observed for one hour, under the influence of the temperature of 20°C. The blankets were then restored for a period of one hour. They were again removed and an ice bag applied to the back of the neck, covering an area of about 60 square centimeters; after 15 minutes the ice bag was removed and the subject then observed for one final hour at the temperature of 20°C.

Skin temperatures were recorded at four points, the ball of the index finger of the right hand, the upper arm, the chest and the side of the calf (the last three, of course, under the clothing), by thermocouples held in contact with the skin by a single strip of adhesive tape.

The results as plotted in figure 5 are striking. It will be noted that the skin temperature fell in all cases on the first exposure to 20°C. but that the additional local and temporary stimulation of the ice pack caused, in each case, a much greater fall and a fall which continued progressively for at least one hour after the cold stimulus was removed. The extent of variation in the case of the finger was, as might be expected, enormously greater than for other areas and somewhat greater in the case of the calf than for the arm and chest.

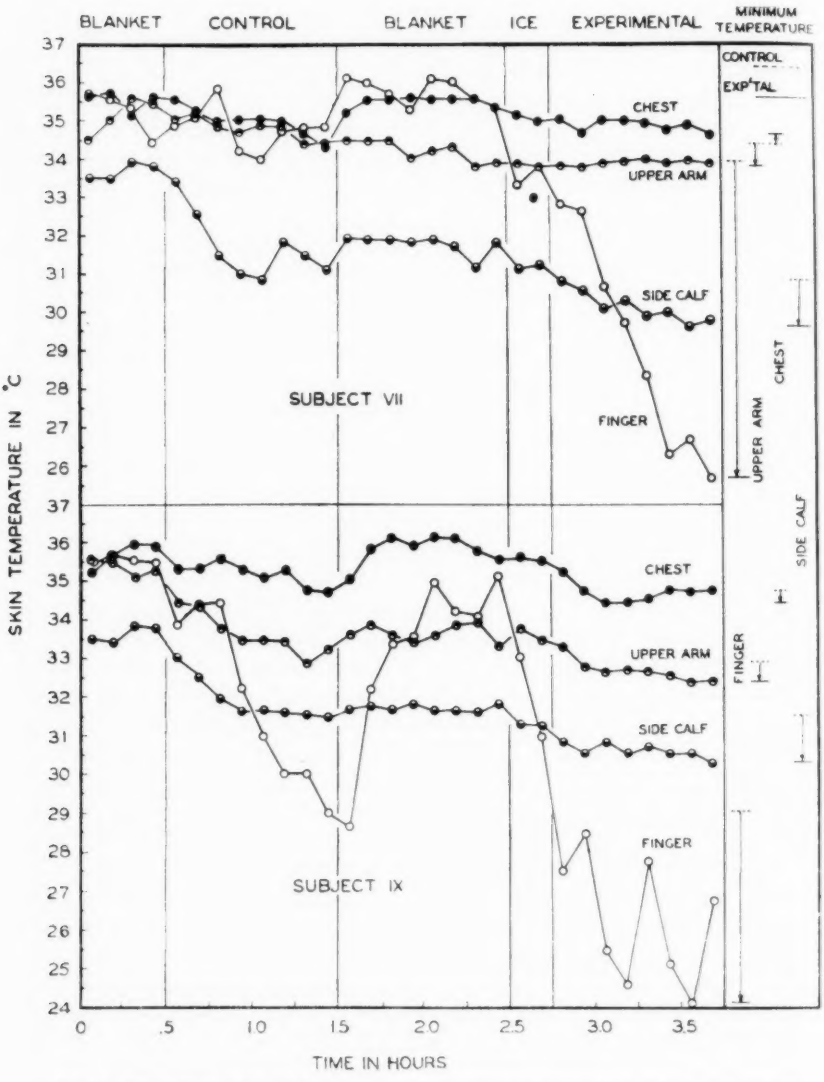



Fig. 5. The effect of a cold stimulus on vasomotor control of skin temperature for the index finger, the upper arm, the chest, and the side calf.

## CONCLUSIONS

1. It appears from these studies that when a given operative temperature is produced by (*a*), air and walls of approximately the same temperature, and (*b*), cooler air and warmer walls, the physiological reactions of the clothed human body differ in the two cases, as follows:

a. Mean skin temperatures are lower for the cold-air warm-wall situation, chiefly as a result of lower skin temperatures for upper and lower extremities, the head and trunk temperatures being less affected.

b. Conductance of the skin is very much lower for the cold-air warm-wall situation, particularly in the hot region.

c. As a result of higher skin temperatures, negative storage (heating of the body) is greater in the hot region, and positive storage (chilling of the body) is less in the cold region, for the cold-air warm-wall situation.

d. Wetted area is less for the cold-air hot-wall situation in the hot region.

e. As a result of the physiological reactions noted above, discomfort is less in the hot region and greater in the cold region for the cold-air warm-wall situation.

2. It is of special interest to note that our curves for conductance in the cold region level off at distinctly higher values for the equal wall and air situation than for the hot-wall cold-air conditions, and these differences in conductance must be related to equally significant differences in the depth of gradient, as suggested in an earlier communication (Gagge, Winslow, and Herrington, 1938). This seems to us to imply possible modification in common assumptions with regard to the phenomena of vasoconstriction. It is ordinarily assumed that vasoconstriction is an "all or none" phenomenon, and that after constriction occurs, the body behaves like a purely physical mechanism. The results here presented strongly suggest that far down in the cold region there are still fundamental physiological regulative forces at work.

## REFERENCES

GAGGE, A. P. This Journal **120:** 277, 1937.

GAGGE, A. P., L. P. HERRINGTON AND C.-E. A. WINSLOW. Am. J. Hyg. **26:** 84, 1937.

GAGGE, A. P., C.-E. A. WINSLOW AND L. P. HERRINGTON. This Journal **124:** 30, 1938.

HARDY, J. D. AND C. MUSCHENHEIM. J. Clin. Invest. **13:** 817, 1934.

HARDY, J. D. AND T. W. OPPEL. Proc. Am. Physiol. Soc. 50th Annual Meeting, 89–90.

HERRINGTON, L. P., C.-E. A. WINSLOW AND A. P. GAGGE. This Journal **120:** 133, 1937.

WINSLOW, C.-E. A., L. P. HERRINGTON AND A. P. GAGGE. This Journal **116:** 669, 1936.

This Journal **120:** 1, 1937a.

Am. J. Hyg. **26:** 103, 1937b.

# THE INFLUENCE OF PIPERIDINOMETHYLBENZODIOXANE (933F) ON THE ACTION OF SYMPATHIN

K. LISSÁK[1]

*From the Department of Physiology in the Harvard Medical School*

Received for publication June 16, 1938

In a previous paper from this Laboratory (Morison and Lissák, 1938) evidence was presented that in the submaxillary gland of the cat—in which the nerve endings are extracellular—the dioxane derivative 933F markedly reduces the responses of the salivary gland to injected adrenalin, without comparably affecting the responses to sympathetic nerve stimuli. Furthermore, 933F was found to increase the rate of oxidation of adrenalin *in vitro* and *in vivo*. Recently Luco and Lissák (1938) pointed out that 933F does not decrease the diffusion of sympathin into the aqueous humor of the cat. It was also shown that these observations made it difficult to explain the action of 933F by changes of cellular permeability (Rosenblueth and Cannon, 1936). It is probable, however, that 933F cannot inhibit the sympathin released inside or very close to the cells rapidly enough to affect significantly the responses to nerve impulses. But when sympathin or adrenalin comes to the cells from distant sources (Bacq and Fredericq, 1935) via the blood stream, 933F has a longer opportunity to destroy the sympathin or adrenalin and consequently to inhibit their action.

In order to establish this explanation more fully the following experiments were undertaken.

METHOD. Hearts of spring frogs (March through May) were prepared for perfusion according to the method of Straub. Standard Ringer's solution was used. In all cases the hearts were rendered hypodynamic by frequent washings with Ringer's solution before the experimental procedures were carried out. In cats under dial anesthesia (0.7 cc. per kgm.) the left cervical sympathetic nerve was isolated, and placed on shielded silver electrodes. Conduction centrally was prevented by crushing. The stimulating current was supplied by a Harvard inductorium, with a secondary coil distance of 5 cm. and a primary current of 5 volts. The drugs used were adrenalin (Parke, Davis), atropine sulphate (Merck), cocaine hydrochloride (Merck) and 933F (kindly supplied by Dr. D. Bovet).

[1] Fellow of the Rockefeller Foundation.

RESULTS. A. *The influence of 933F on the sympathin of the frog's heart.* Two hypodynamic frog hearts were used. The donor heart (D) was perfused with Ringer's solution containing atropine (1:100,000) to prevent the effect of the acetylcholine which was liberated, and the vagosympathetic trunk was stimulated during the perfusion. The sympathetic stimulation caused a marked increase in the amplitude of the contractions of heart D (fig. 1, interval *a* to *b*). After the stimulation the perfusate was removed and was transferred to the recipient heart (R), which had previously been washed with atropinized Ringer's solution. A marked positive inotropic effect on the heart R ensued (fig. 2*a*; Loewi, 1921, 1922). If the same perfusate from the donor heart D was removed and 933F added to give a concentration of 1:100,000 before transference to the

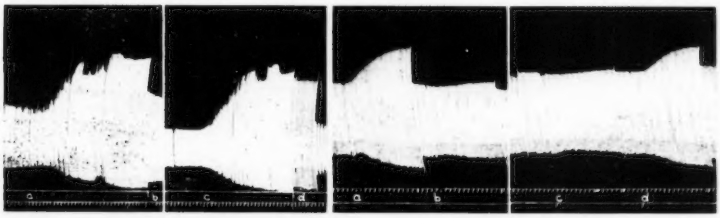



Fig. 1 Fig. 2

Fig. 1. Donor heart D. Hypodynamic, isolated from the frog. In this and the succeeding records the time signal marks 5-second intervals.

During interval *a* to *b*, Ringer's solution with atropine (1:100,000), stimulation of the vagosympathetic trunk; during *c* to *d*, Ringer's solution with atropine and 933F (1:100,000), stimulation of the vagosympathetic trunk.

Fig. 2. Recipient heart R. At *a*, perfusate from heart D (fig. 1*a* to *b*); at *b*, same as at *a*, but with 933F (1:100,000); at *c*, perfusate from heart D (fig. 1*c* to *d*); at *d*, adrenalin (1:1,000,000,000).

recipient heart R (previously washed with Ringer's solution containing atropine and 933F (1:100,000), the positive inotropic effect was either very small (2 cases) or completely absent (8 cases) (fig. 2*b*).

If the heart D was perfused with Ringer's solution containing atropine and 933F (1:100,000), the vagosympathetic stimulation resulted in about the same increase in the contractions as in the first case (Shen, 1937; fig. 1*c* to *d*), but this perfusate was completely ineffective when added to the heart R, which had previously been washed with atropine-933F Ringer's solution (fig. 2*c*).

B. *The influence of 933F on the sympathin of the aqueous humor of the cat.* In a recent paper from this Laboratory (Luco and Lissák, 1938) it was concluded that in the cocainized cat stimulation of the cervical sympathetic released sympathin to the aqueous humor. The same tech-

nique was employed in the following experiments. In 8 cases, after a 10-minute stimulation of the cervical sympathetic on one side, the aqueous humor was removed on both the stimulated and the normal side. Each fluid was diluted 1:3 with Ringer's solution and tested first on a hypodynamic frog's heart, and second on a heart previously washed with 933F (1:100,000). Such an experiment is illustrated in figure 3. For quantitative comparison titrations with adrenalin were made on the same heart. The aqueous humor from the stimulated side had a marked positive inotropic effect on the frog's heart approximately equal to that produced by a 1:500,000,000 dilution of adrenalin (fig. 3*a*, *c*, *d*). The aqueous humor from the opposite non-stimulated side was ineffective (fig. 3*b*). On the heart treated with 933F the aqueous humor from the stimulated side had a very small positive effect, adrenalin 1:1,000,000,000 was ineffective and 1:500,000,000 gave a small reaction (fig. 3*b*, *e*, *f*, *g*, *h*).

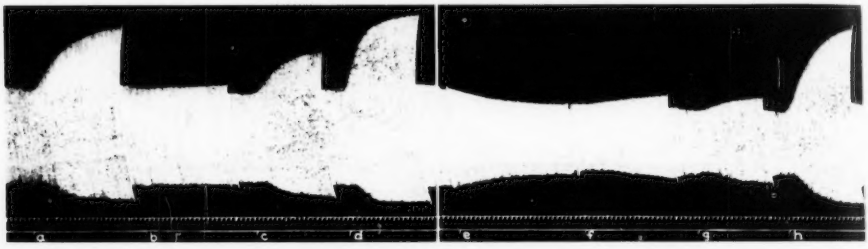



Fig. 3. Heart R. At *a*, aqueous humor from the stimulated side (1:3); at *b*, aqueous humor from the normal side; at *c*, adrenalin (1:1,000,000,000); at *d*, adrenalin (1:100,000,000); at *e*, 933F (1:100,000); at *f*, same as at *a*; at *g*, adrenalin (1:500,000,000); at *h*, same as at *d*.

If 933F (1:100,000) was added to the aqueous humor *in vitro* and oxygen was bubbled through it for one hour, the positively acting substance was destroyed, the solution being ineffective on a heart washed with 933F. Control experiments showed that oxygenated aqueous humor, left at the laboratory temperature, was still active 5 to 6 hours after it was collected from the animal. Bonhomme (1936) reported that the aqueous humor has an antioxidative power on adrenalin.

Discussion. Morison and Lissák (1938) reported, in disagreement with Shen (1937), that 933F does not significantly inhibit the stimulant effect of adrenalin on the winter frog's heart (November through February) except in rather special circumstances (cf. Hill and Myers, 1938). But in the spring frog's heart (March through May) an interesting seasonal difference appeared. On the winter frog's heart a very high dilution of adrenalin (1:1,000,000,000) was not affected by 933F (1:100,000), but on the spring frog's heart the adrenalin effect is inhibited or reduced by the

same concentration of 933F (fig. 3). This inhibitory effect of 933F is produced only with a limited concentration of adrenalin, which is approximately equal to a physiological sympathin concentration (1:1,000,000,000 to 1:500,000,000), but not with the extremely high concentration (1:80,000) reported by Shen (1937). This latter unphysiologically high adrenalin concentration usually has an inhibitory effect on the hypodynamic frog's heart in any season, even in the absence of 933F.

The experiments reported in sections A and B show that 933F has the same effect on sympathin as it has on adrenalin. It prevents or inhibits the effect of both adrenalin and sympathin without comparably impeding sympathetic stimulation, as has been reported by others. If sympathin is added from another source to the frog's heart, the reaction to sympathin and the inhibition with 933F is the same as with adrenalin. Sympathin is inactivated *in vitro* with 933F as is adrenalin. It may be concluded, therefore, that 933F does not reveal any significant difference between adrenalin and sympathin when contained in a perfusate.

## SUMMARY

1. The positive inotropic effect on a recipient frog's heart perfused with sympathin from another frog's heart or from the aqueous humor of a cat is abolished by 933F (figs. 2 and 3).

2. The response of the donor heart to stimulation of its own sympathetic nerve supply is unaffected by 933F (fig. 1).

3. The destruction of sympathin *in vitro* is accelerated by 933F.

4. These observations render unlikely the view that the difference between the effect of 933F on sympathetic nerve stimulation and on adrenalin is due to a difference between sympathin and adrenalin.

## REFERENCES

Bacq, Z. M. and H. Fredericq. Arch. Internat. Physiol. **41:** 334, 1935.
Bonhomme, F. Ibid. **43:** 341, 1936.
Hill, W. T. and G. S. Myers. Unpublished data, 1938.
Loewi, O. Pflüger's Arch. **189:** 239, 1921.
Ibid. **193:** 201, 1922.
Luco, J. V. and K. Lissák. This Journal **124:** 271, 1938.
Morison, R. and K. Lissák. Ibid. **123:** 404, 1938.
Rosenblueth, A. and W. B. Cannon. Ibid. **116:** 414, 1936.
Shen, T. C. R. Arch. Internat. de Pharmakodyn. et de Therap. **55:** 493, 1937.

# STUDIES ON THE FORMATION OF AMMONIA BY THE KIDNEY

J. W. CAVETT AND W. C. FOSTER

*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis*

Received for publication June 17, 1938

In a small number of experiments in which different individuals were fed the same diet, their urines were found to vary so much in pH that the question arose as to whether there were individual differences in acid formation, or whether some kidneys were more efficient than others in neutralizing the acids excreted. Since the formation of ammonia plays such an important rôle in the regulation and the conservation of fixed bases in the animal body when acid is excreted, it seemed advisable to study the problem from that point of view.

Walter (1) noted that dogs could tolerate hydrochloric acid by stomach tube much better than rabbits. The former showed an increase in urinary ammonia under this condition.

Magnus-Levy (2) showed that diabetics formed large amounts of beta hydroxy butyric acid and that in this acidosis there was an increase in the urine ammonia. Haskins (3) found the urinary ammonia to vary with acid and base-forming elements in the diet. Howe and Hawk (4) studied the ammonia and pH of the urine in a starving man and found no relationship. Benedict (5) compared urinary ammonia and titratable acidity in cases of ketosis and found a variable ammonia-titratable acid ratio which was usually larger than one. Van Slyke and co-workers (6) found the ratio of 0.1 N ammonia to 0.1 N acid to range from 0.7 to 2.8 for normal individuals while in nephritic patients with impaired urea excretion the ratio was always less than one. Briggs (7) found that a moderate diuresis caused by drinking distilled water failed to stimulate ammonia production, thus indicating that more than a loss of fixed base was necessary. Nash and Benedict (8) and also Rabinowich (9) have shown that the kidney formed ammonia for the purpose of neutralizing acids.

EXPERIMENTAL. Urine from individuals who had no pathological condition were used. Pucher and co-workers (10) reported fewer variations in the constituents of morning urine than in 24 hour specimens, so a study on five individuals was carried out. The variations of the urine constituents in first morning specimens from day to day for an individual were quite constant and less than the variation found at different periods during the same day. In order to get more uniform samples and since care had

TABLE 1

*Data from analyses of 36 specimens of first morning urine*

| NUMBER | TIME COLLECTED | IN BLADDER | VOLUME | SPECIFIC GRAVITY | x pH | y TITRATABLE ACIDITY cc. N/10 NaOH PER 100 cc. URINE | z AMMONIA EXPRESSED AS cc. N/10 PER 100 cc. URINE | UREA N GRAM. 100 cc. URINE | ORIGINAL UREA CHANGED TO AMMONIA | a ACID NEUTRALIZED BY AMMONIA |
|---|---|---|---|---|---|---|---|---|---|---|
| | *a.m.* | *hours* | *cc.* | | | | | | *per cent* | *per cent* |
| 32 | 8:00 | 8 | 276 | 1.009 | 5.27 | 16 | 17 | 0.61 | 3.9 | 51 |
| 35 | 8:30 | 9 | 551 | 1.008 | 5.41 | 16 | 21 | 0.51 | 5.9 | 56 |
| *90 | 7:30 | 6.5 | 181 | 1.015 | 6.15 | 27 | 23 | 0.82 | 3.9 | 45 |
| *80 | 7:15 | 6.25 | 210 | 1.009 | 5.64 | 17 | 26 | 0.68 | 5.0 | 60 |
| *88 | 8:00 | 6 | 195 | 1.023 | 5.41 | 20 | 29 | 0.81 | 4.8 | 58 |
| 75 | 8:00 | 8 | 186 | 1.016 | 5.92 | 33 | 30 | 1.32 | 3.1 | 47 |
| 19 | 8:00 | 7 | 195 | 1.025 | 5.52 | 24 | 32 | 0.90 | 4.9 | 57 |
| *53 | 8:30 | 8 | 210 | 1.012 | 5.35 | 27 | 33 | 0.83 | 5.5 | 54 |
| *76 | 7:45 | 8.5 | 60 | 1.016 | 6.32 | 10 | 33 | 0.80 | 5.4 | 76 |
| *54 | 8:15 | 9 | 195 | 1.027 | 5.55 | 36 | 34 | 1.50 | 3.1 | 48 |
| *77 | 7:30 | 8 | 160 | 1.025 | 5.72 | 31 | 35 | 1.24 | 3.9 | 53 |
| 27 | 7:00 | 7.5 | 215 | 1.013 | 5.50 | 23 | 37 | 0.83 | 6.2 | 61 |
| 30 | 8:00 | 9 | 214 | 1.030 | 5.92 | 37 | 37 | 1.64 | 3.2 | 48 |
| *71 | 8:30 | 8.5 | 205 | 1.023 | 5.41 | 47 | 39 | 1.44 | 3.7 | 45 |
| *82 | 7:00 | 6 | 187 | 1.017 | 5.01 | 39 | 41 | 1.27 | 4.3 | 51 |
| *83 | 7:00 | 6 | 200 | 1.014 | 5.71 | 27 | 41 | 0.69 | 7.7 | 60 |
| *84 | 8:30 | 8.5 | 156 | 1.018 | 5.49 | 43 | 41 | 1.07 | 5.1 | 48 |
| *91 | 7:30 | 8.5 | 126 | 1.026 | 5.59 | 46 | 41 | 1.31 | 4.2 | 47 |
| 100 | 7:00 | 8 | 260 | 1.031 | 5.34 | 37 | 41 | 1.34 | 4.1 | 52 |
| 3 | 6:30 | 7 | 175 | 1.026 | 5.25 | 35 | 42 | 1.35 | 4.3 | 54 |
| *78 | 7:30 | 8.5 | 115 | 1.021 | 5.80 | 31 | 43 | 0.99 | 5.9 | 58 |
| *79 | 8:00 | 7 | 153 | 1.023 | 5.59 | 46 | 44 | 1.30 | 4.6 | 49 |
| 47 | 6:00 | 7 | 226 | 1.025 | 5.29 | 39 | 44 | 1.61 | 3.8 | 53 |
| *85 | 8:00 | 6 | 193 | 1.021 | 5.65 | 33 | 45 | 1.44 | 4.2 | 56 |
| *74 | 8:00 | 6 | 152 | 1.018 | 5.79 | 29 | 46 | 1.06 | 5.7 | 61 |
| 49 | 7:00 | 8 | 293 | 1.028 | 5.34 | 48 | 50 | 1.70 | 4.1 | 51 |
| *52 | 8:30 | 8 | 158 | 1.028 | 5.07 | 70 | 55 | 1.44 | 5.3 | 44 |
| *86 | 8:30 | 7.25 | 180 | 1.023 | 5.01 | 58 | 57 | 1.38 | 5.4 | 49 |
| *45 | 5:45 | 7 | 172 | 1.028 | 5.39 | 58 | 61 | 1.93 | 4.4 | 51 |
| *55 | 8:30 | 7.5 | 150 | 1.029 | 5.60 | 40 | 66 | 1.70 | 5.3 | 62 |
| 44 | 6:35 | 8 | 170 | 1.027 | 5.21 | 64 | 77 | 1.83 | 5.9 | 54 |
| *73 | 8:00 | 7 | 126 | 1.017 | 5.10 | 82 | 81 | 1.90 | 5.6 | 49 |
| *81 | 7:30 | 8 | 185 | 1.021 | 4.91 | 63 | 82 | 1.56 | 6.8 | 56 |
| *87 | 8:30 | 7 | 165 | 1.026 | 5.21 | 65 | 82 | 1.73 | 6.3 | 55 |
| 23 | 7:00 | 7 | 185 | 1.031 | 5.45 | 46 | 88 | 1.45 | 8.5 | 65 |
| *70 | 7:30 | 8 | 174 | 1.030 | 5.35 | 81 | 108 | 1.72 | 8.1 | 57 |

* F.E.R.A. students who were maintained on diets in which the chief variation was in the quantity of food eaten.

to be taken to prevent loss of carbon dioxide or the production of ammonia by bacteria, it was decided to use only the first morning sample.

Urine was collected in sterilized bottles which contained a few drops of toluene to prevent any possible formation of ammonia by bacterial action. To insure against the loss of $CO_2$ which would affect the pH, one hundred cubic centimeters of the sample were immediately placed in a sterilized 100 cc. rubber-stoppered bottle. The urine was then refrigerated until analyzed, one to three hours later. The volume, specific gravity, pH, titratable acidity, ammonia and urea were determined and the per cent of acid neutralized by ammonia and the per cent of original urea changed to ammonia calculated.

Urea was determined by the method of Van Slyke and Cullen (11) using urease, followed by aeration and titration. The value resulting included urea plus ammonia. The ammonia was determined in a similar manner

TABLE 2

*Statistics*

| | *x* pH | *y* TITRATABLE ACIDITY | *z* AMMONIA | *b* TOTAL ACIDITY | *a* PER CENT ACID NEUTRALIZED BY AMMONIA |
|---|---|---|---|---|---|
| Mean | 5.478889 | 40.111111 | 47.555555 | 87.388889 | 53.916667 |
| Maximum | 6.32 | 82. | 108. | 189. | 76. |
| Minimum | 4.91 | 10. | 17. | 33. | 44. |
| Standard deviation | 0.307584 | 17.816624 | 19.951950 | 36.897965 | 6.426405 |

*Correlation coefficients*

| | TITRATABLE ACIDITY (*y*) | AMMONIA (*z*) | TOTAL ACIDITY (*b*) | PER CENT ACID NEUTRALIZED BY AMMONIA (*a*) |
|---|---|---|---|---|
| pH (*x*) correlated with | $r_{xy} = -0.579288$ | $r_{xz} = -0.708945$ | $r_{xb} = -0.528974$ | $r_{xa} = +0.304505$ |
| Relative deviate | $x' = 3.44$ | $x' = 4.19$ | $x' = 3.13$ | $x' = 1.80$ |
| Ammonia (*z*) correlated with | $r_{yz} = 0.840170$ | | $r_{zb} = 0.964362$ | |
| Relative deviate | $x' = 4.97$ | | | |
| Influence of pH removed | $r_{yz.x} = 0.747091$ | | | |

except no urease was added before the aeration. A glass electrode (12) was used for the pH determination. The method of Henderson and Palmer (13) was used to determine titratable acidity. This method was chosen because the pH of its end point is that of blood (7.4) and because only about 1 per cent of the ammonia salts are titrated with sodium hydroxide at this pH. The specific gravity was determined with a urinometer. The data for the various determinations are presented in table 1.

The statistics presented in table 2 were derived with the following formulae (14).

The mean, $\bar{x} = \frac{\Sigma x}{N}$ where $\Sigma x$ is the sum of the variates and $N$ is the number of variates.

Standard deviation,

$$\sigma x = \sqrt{\frac{\Sigma x^2}{N} - \bar{x}^2}$$

$\Sigma x^2$ is the sum of the squares of each variate and $\bar{x}^2$ the square of the mean. The correlation coefficient

$$r_{xy} = \frac{\frac{\Sigma xy}{N} - \bar{x}\bar{y}}{\sigma^x \sigma^y}$$

$\Sigma xy$ is the sum of the products of the pairs of variates.

The residual correlation equation,

$$r_{yz.x} = \frac{r_{yz} - r_{xy} r_{xz}}{\sqrt{(1 - r_{xy}^2)(1 - r_{xz}^2)}}$$

used to correlate between two variates with the influence of a third variate removed.

Relative deviate, $x' = r\sqrt{N - 1}$ where $r$ is the correlation coefficient of a pair of variates and $N$ is the number of variates. A value greater than 2 is considered as a significant correlation.

Discussion. The data obtained from analyses of 36 specimens of first morning urine are presented in table 1. There does not appear to be any definite relation between the volume, specific gravity, urea nitrogen and the per cent of acid neutralized by the ammonia. In this study the acid neutralized by fixed base is not considered, thus the term acid includes only the titratable acidity plus the acid neutralized by the ammonia. Both are expressed as cubic centimeters of 0.1 normal solution per 100 cc. of urine. About 1 per cent of the ammonia salt present will react with sodium hydroxide in determining titratable acidity by the method used. This affected the data so little that no correction was made.

It became apparent that a statistical study of the data for pH, titratable acidity, per cent of acid neutralized by ammonia and ammonia content was necessary. The statistics are presented in table 2.

The correlation coefficient for titratable acidity and pH is $-0.58$. This indicates that the titratable acidity of the urine tends to vary inversely with the pH and is of some significance since the relative deviate is 3.44. One could hardly expect a high correlation, for a urine with a given pH which contains greater quantities of the more highly ionized acids and less of the slightly ionized acids will have a lower titratable acidity value than another with the same pH value which contains a greater per cent of slightly ionized acids.

Therefore it is possible for a urine with a low pH to show a lower titratable acidity value than another with a higher pH (i.e., 32 and 90). (Table 1.)

The ammonia content has a somewhat closer relation to the pH as is shown by a correlation coefficient of −0.71. However, the ammonia content of urine was found more closely related to the titratable acidity as this gives a correlation coefficient of 0.84. The correlation coefficient of ammonia and titratable acidity was still 0.75 when the influence of pH was removed. If the correlation coefficient had remained 0.84 it would have indicated that pH had no influence on the ammonia content but if pH had been entirely responsible, the correlation coefficient would have become zero. The rather high correlation coefficient of 0.96 when ammonia content is correlated with total acid (titratable acidity plus acid neutralized by ammonia) also agrees with the above findings and together they indicate that the ammonia content of urine is more dependent upon the amount of acid radicals present than upon the pH of the urine.

The per cent of acid neutralized by ammonia varied among the 36 individuals from 44 to 76 per cent, having a mean value of 53.9 and a standard deviation of 6.43.

When five individuals were studied, the greatest day to day variation found for the per cent of acid neutralized by ammonia in the morning specimen of urine was 7 per cent. Two to six determinations were made for each individual. The variation from hour to hour during the day for these same individuals was several times as great.

The per cent of acid neutralized by ammonia does not appear to be dependent upon the urinary constituents studied. The pH gave a correlation coefficient of only 0.30 which is insignificant. Very likely the per cent of acid neutralized by ammonia depends upon the ability of the individual kidney to form ammonia. The reports of Van Slyke and co-workers (6) and Briggs (7) on nephritic kidneys and their reduced ability to form ammonia would also suggest that normal kidneys might have an individual variation in their ability to form ammonia. This ability to form ammonia does not appear to be responsible for the variations in the pH of the urine from individuals on similar diets as can be seen by the data of table 1.

## SUMMARY

The data of analysis on 36 specimens of first morning urine for volume, specific gravity, urea nitrogen, ammonia nitrogen, titratable acidity, and pH have been presented.

A statistical study of the data for pH, titratable acidity, ammonia nitrogen and the per cent of acid neutralized by ammonia has been made. The correlation coefficient of pH with titratable acidity was −0.58; pH with ammonia content, −0.71; pH with the per cent of acid neutralized

by ammonia 0.30; ammonia content with titratable acid 0.84. When the influence of pH was removed the correlation coefficient of ammonia content to titratable acidity was still 0.75. The correlation coefficient of ammonia and total acid (titratable acidity plus that neutralized by the ammonia) was 0.96.

These data indicate that ammonia formation by the kidney is more dependent upon titratable acidity, and especially the amount of acid material present to be neutralized than upon the pH of the urine.

The per cent of acid neutralized by ammonia appears to be dependent upon the individual kidney rather than any of the components studied.

## REFERENCES

(1) Walter, F. Arch. f. Exper. Path. u. Pharmakol. **7:** 148, 1877.
(2) Magnus-Levy, A. Arch. f. Exper. Path. u. Pharmakol. **42:** 149, 1899.
(3) Haskins, H. D. J. Biol. Chem. **2:** 217, 1906 07.
(4) Howe, P. E. and P. B. Hawk. J. Biol. Chem. **17:** 48, 1914.
(5) Benedict, F. G. Nutrition Lab., Carnegie Inst. Wash. Pub. no. 203, 416, 1915.
(6) Van Slyke, D. D., G. C. Linder, A. Hiller, L. Leiter and J. F. McIntosh. J. Clin. Invest. **2:** 255, 1926.
(7) Briggs, A. P. Arch. Int. Med. **49:** 56, 1932.
(8) Nash, T. P., Jr and S. R. Benedict. J. Biol. Chem. **48:** 463, 1921.
(9) Rabinowitch, I. M. Arch. Int. Med. **33:** 394, 1924.
(10) Pucher, G. W., F. R. Griffith, K. A. Brownell, J. D. Klein and M. E. Carmer. J. Nutrition **7:** 131, 1934.
(11) Van Slyke, D. D. and G. E. Cullen. J. Biol. Chem. **24:** 117, 1916.
(12) Hemingway, A. Ind. Eng. Chem. **7:** 203, 1935.
(13) Henderson, L. J. and W. W. Palmer. J. Biol. Chem. **17:** 305, 1914.
(14) Treloar, A. E. An outline of biometric analysis. Burgess Publishing Co., Minneapolis, 1935.

# POTASSIUM CHANGES IN SUBMAXILLARY GLANDS DURING STIMULATION

J. H. WILLS AND W. O. FENN

*From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.*

Received for publication June 17, 1938

Numerous studies have been made of the electrolyte content of the saliva, but so far as we have been able to discover, the electrolyte content of the gland itself as affected by secretion has never been investigated. We are reporting here some analyses for potasium in resting and stimulated submaxillary glands of cats. Potassium seemed to be a particularly important element to investigate in this regard because of the relative constancy of its concentration in the saliva under varying conditions. Moreover, since it has been found that muscles lose potassium during stimulation it would be interesting to know whether or not glands behave similarly during activity.

EXPERIMENTAL. The submaxillary glands of cats were used for all the work here reported, the general procedure of the experiments being essentially that used by Pavlov (1888), Henderson (1900), and Anrep (1921) in their work on the nitrogen change in salivary glands during stimulation. The potassium analyses were done by the method used by Fenn and Cobb (1936).

The following protocol will illustrate the general form of the experiments:

2/2/37—Cat, weight 2.6 kgm., anesthetised with Dial at 9:30 a.m. The duct of the left submaxillary gland was exposed and a glass cannula inserted therein. The lingual nerve of this side was then cut as close as possible to the tympanic bulla, and the peripheral section arranged for stimulation by a thyratron stimulator through platinum electrodes. Stimulation at a frequency of 15 per sec. was commenced at 10:30 a.m., the saliva being collected in a tared weighing bottle. After approximately 25 minutes the animal's thorax was opened and the aorta sectioned. Salivary flow ceased at 11:00 a.m., giving a total secretion time of 30 minutes. The stimulated gland and the control gland from the right side of the cat were removed as rapidly as possible, carefully freed of extraneous tissue and blood, and placed in tared weighing bottles. After drying to constant weight at 100°C., the glands and saliva were analysed for potassium.

In the experiments in which pilocarpine stimulation was used, the nerve was not dissected out as above but a cannula was inserted into one femoral vein for injection of the drug, and the control gland was excised before commencement of stimulation.

## TABLE 1

*Data of experiments*

| STIMULATION | WEIGHT OF CAT | STIMULATION | SUBMAXILLARY GLAND | | | | | | SALIVA | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Wet Weight | | Dry Weight | | Potassium | | Secreted per gram dry weight of gland per hour | Potassium |
| | | | Control | Stimulation | Control | Stimulation | Control | Stimulation | | |
| | *kgm.* | *minutes* | *grams* | *grams* | *grams* | *grams* | *mM/100 gm. dry wt.* | *mM/100 gm. dry wt.* | *cc.* | *mM/100 cc.* |
| Electrical | 2.6 | 30 | 1.107 | 1.265 | 0.288 | 0.314 | 36.7 | 37.2 | 12.4 | 1.60 |
| | 2.6 | 44 | 1.227 | 1.197 | 0.280 | 0.246 | 35.4 | 34.6 | 21.6 | 1.04 |
| | 1.7 | 40 | 0.879 | 1.231 | 0.198 | 0.203 | 34.9 | 39.8 | 16.3 | 1.05 |
| | 4.6 | 45 | 1.242 | 1.491 | 0.316 | 0.339 | 31.8 | 34.4 | 16.2 | 0.75 |
| | 3.7 | 37 | 1.150 | 1.463 | 0.282 | 0.308 | 33.0 | 34.1 | 18.8 | 1.55 |
| | 2.2 | 45 | 0.871 | 0.793 | 0.215 | 0.198 | 34.6 | 33.2 | 44.4 | 2.18 |
| | | 30 | 1.268 | 1.231 | 0.295 | 0.272 | 37.4 | 35.6 | 18.9 | 0.87 |
| | 3.0 | 60 | 1.212 | 1.325 | 0.302 | 0.311 | 30.8 | 28.2 | 17.5 | 0.69 |
| | 3.2 | 58 | 1.222 | 1.306 | 0.272 | 0.252 | 38.0 | 37.4 | 23.6 | 0.73 |
| | 4.2 | 67 | 1.742 | 1.961 | 0.413 | 0.393 | 35.3 | 34.7 | 23.4 | 0.97 |
| | 2.6 | 39 | 0.934 | 0.966 | 0.234 | 0.213 | 29.6 | 31.9 | 62.2 | 0.76 |
| | 1.1 | 30 | 0.751 | 0.800 | 0.174 | 0.171 | 41.3 | 42.7 | 87.3 | 0.76 |
| | 1.7 | 30 | 0.728 | 0.786 | 0.186 | 0.150 | 28.8 | 35.2 | 126.3 | 0.62 |
| | 1.5 | 30 | 0.688 | 0.843 | 0.181 | 0.166 | 30.5 | 33.6 | 39.1 | 0.71 |
| | 2.3 | 30 | 0.973 | 1.246 | 0.255 | 0.250 | 38.8 | 32.6 | 51.6 | 0.69 |
| | 2.4 | 33 | 1.095 | 1.011 | 0.285 | 0.273 | 32.0 | 29.6 | 48.5 | 0.72 |
| | 3.4 | 33 | 1.290 | 1.271 | 0.307 | 0.267 | 40.9 | 43.3 | 88.5 | 0.81 |
| | 3.5 | 35 | 1.007 | 0.890 | 0.250 | 0.234 | 35.8 | 32.4 | 81.2 | 0.51 |
| | 2.4 | 33 | 0.794 | 0.710 | 0.189 | 0.172 | 41.8 | 37.8 | 68.5 | 0.78 |
| | 1.8 | 38 | 1.157 | 1.164 | 0.282 | 0.257 | 37.7 | 40.3 | 31.2 | 0.80 |
| | 1.8 | 31 | 1.091 | 1.146 | 0.256 | 0.234 | 43.6 | 43.7 | 58.4 | 0.68 |
| | 3.6 | 37 | 1.472 | 1.507 | 0.359 | 0.311 | 29.8 | 32.8 | 31.2 | 0.99 |
| | 2.5* | 31 | 1.205 | 1.259 | 0.300 | 0.280 | 34.4 | 31.8 | 55.2 | 0.55 |
| | 2.8* | 35 | 1.652 | 1.882 | 0.364 | 0.321 | 36.3 | 47.2 | 45.0 | 0.65 |
| Pilocarpine | 3.2 | 16 | 1.709 | 1.523 | 0.369 | 0.337 | 42.6 | 29.2 | 63.3 | 0.85 |
| | 3.0 | 17 | 1.302 | 1.275 | 0.317 | 0.316 | 35.2 | 21.2 | 62.0 | 1.02 |
| | 2.6 | 44 | 1.182 | 1.480 | 0.279 | 0.269 | 34.6 | 25.4 | 36.8 | 0.87 |
| | 2.6 | 56 | 1.095 | 0.969 | 0.271 | 0.218 | 34.4 | 22.7 | 42.6 | 0.83 |
| | 2.6 | 65 | 1.139 | 1.214 | 0.281 | 0.283 | 32.6 | 31.2 | 25.0 | 0.88 |
| | 2.5 | 36 | 1.035 | 0.826 | 0.254 | 0.244 | 37.4 | 22.5 | 65.9 | 0.75 |
| | 2.1 | 31 | 0.670 | 0.742 | 0.167 | 0.207 | 36.2 | 22.8 | 79.0 | 0.87 |
| | 2.4 | 31 | 1.217 | 0.963 | 0.293 | 0.277 | 28.2 | 24.3 | 43.2 | 0.75 |
| | 3.2 | 39 | 1.294 | 1.386 | 0.325 | 0.382 | 29.8 | 31.2 | 19.2 | 1.04 |
| | 2.9 | 32 | 1.020 | 0.936 | 0.276 | 0.270 | 30.9 | 24.8 | 33.1 | 0.64 |
| | 2.2 | 30 | 1.051 | 0.958 | 0.255 | 0.237 | 29.5 | 22.4 | 51.8 | 0.63 |
| | 2.3 | 48 | 1.050 | 0.895 | 0.261 | 0.240 | 33.4 | 21.8 | 50.4 | 0.63 |
| | 3.0 | 44 | 1.120 | 1.098 | 0.280 | 0.273 | 29.4 | 27.9 | 16.2 | 0.82 |
| | 2.4* | 30 | 1.030 | 0.993 | 0.257 | 0.261 | 34.4 | 26.2 | 47.1 | 0.65 |
| | 2.6* | 32 | 0.918 | 0.843 | 0.249 | 0.243 | 31.8 | 24.6 | 33.0 | 0.65 |

* Decerebrate.

Right and left submaxillary glands of the cats were stimulated alternately in successive experiments. The rate of secretion was varied by changing either the strength of the shock or the amount of pilocarpine given, as the case might be. Two cats in the series of experiments with electrical stimulation and two in the pilocarpine series were decerebrated under ether anesthesia, and were allowed to recover from the operation for several hours before being used. The data from these animals agreed so well with those obtained from the cats under Dial anesthesia that they were included in the same table.

Table 1 gives the data obtained from 24 experiments with electrical stimulation and from 15 in which pilocarpine stimulation was employed. In all, 32 experiments with electrical stimulation were performed, eight of these being discarded because of obvious technical errors.

TABLE 2

*Summary of water and potassium in submaxillary gland during stimulation (per 100 grams dry wt. of gland)*

| STIMULATION | NUMBER OF EXPERIMENTS | | WATER | | POTASSIUM | | TURNOVER OF K | | SECRETION OF SALIVA |
|---|---|---|---|---|---|---|---|---|---|
| | | | Control | Stimulation | Control | Stimulation | Intake from blood | Output in saliva | |
| | | | *cc.* | *cc.* | *mM* | *mM* | *mM per hour* | *mM per hour* | *cc. per hour* |
| Electrical | 24 | Mean | 312 | 369 | 35.4 | 36.0 | 36.7 | 34.9 | 4530 |
| | | $\sigma$ | 21 | 56 | 4.0 | 4.6 | 19.7 | 17.8 | 2850 |
| Pilocarpine | 15 | Mean | 305 | 296 | 33.4 | 25.4 | 18.1 | 35.5 | 4460 |
| | | $\sigma$ | 21 | 54 | 3.5 | 3.1 | 8.8 | 15.6 | 1730 |

$\sigma$ = standard deviation.

Table 2 summarizes the water and potassium contents of the submaxillary gland before and after secretion, with either electrical or pilocarpine stimulation. The intake of potassium from the blood and the output in the saliva were calculated from the figures in table 1 by use of the following formulae:

$$\text{Intake of K in mM per 100 grams dry weight of gland per hour} = \frac{60G(\triangle G + s)}{Wt}$$

$$\text{Output of K in mM per 100 grams dry weight of gland per hour} = VS$$

Here $G$ is the total mM of K initially present in the stimulated gland calculated from the K content of the control gland on the basis of the dry weights of the two glands, $\Delta G$ is the change of K in the gland during stimulation (i.e., the difference between $G$ and the actual K content of the stimulated gland at the conclusion of the experiment) in per cent of $G$, $s$ repre-

sents the amount of K secreted in the saliva during the experiment in per cent of $G$, $W$ is the dry weight of the stimulated gland in grams, $t$ is the duration of secretion in minutes, $V$ represents the cubic centimeters of saliva secreted per gram dry weight of the gland per hour, and $S$ is the mM of K per 100 cc. of saliva.

It will be seen from table 2 that in the series of experiments in which the gland was stimulated through the nerve there was no significant change in any of the quantities measured as a result of the activity of the gland. The control glands contained 35.4 mM of K per 100 grams dry weight and the stimulated glands contained 36.0. Consequently, there was no significant difference between the amount of potassium secreted by the gland

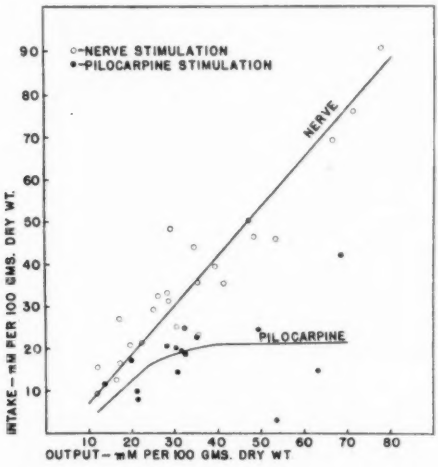



Fig. 1. Variation of intake of potassium by the submaxillary gland with the output in the saliva, with electrical and pilocarpine stimulation.

in the saliva and the amount taken in by the gland during secretion. The variation of water content on the stimulated side was much greater than on the control side.

In the experiments with pilocarpine stimulation there was no significant change in the water content of the gland during secretion, but the potassium content of the stimulated gland decreased from 33.4 to 25.4 mM per 100 grams dry weight although there was no greater production of saliva than before and this saliva contained a normal amount of potassium. This indicates an insufficient intake of potassium from the blood, the calculated intake by the gland being only one-half the output in the saliva.

In figure 1 the intake of potassium by the gland is plotted against the output in the saliva during secretion. It will be seen that in the experi-

ments with electrical stimulation the intake kept pace with the output, giving a reasonably good straight line. However, in the experiments with pilocarpine stimulation there was agreement between the intake and output only at the lower levels of output; with larger outputs the intake lagged behind.

The concentration of potassium in the saliva has been found to be remarkably constant whether electrical or pilocarpine stimulation is used (Gregersen, 1931) and at various rates of flow (Gregersen, 1931; Brown and Klotz, 1937). However, Wach (1936) found rather large variations in the potassium contents of saliva from the same subjects at different times, and from different subjects. The reason for these variations is not apparent. Under the conditions of our work the potassium content of the saliva was generally quite constant, and this constancy seems to indicate that potassium is an essential part of the saliva. It is as though some constant constituent of saliva, itself an intracellular product but presumably not mucin (Wach, 1936), was necessarily put out in combination with the chief intracellular base, potassium. If this were the case, it might be predicted that secretion under the influence of pilocarpine would cease when the potassium supply in the gland ran out, since we have found that the gland stimulated with pilocarpine is not able to replace completely the potassium lost in the saliva during stimulation. The rate of secretion with pilocarpine stimulation does diminish with time, as is well known, but whether or not potassium is a limiting factor in this decrease remains for future investigation to decide.

## SUMMARY

Submaxillary glands of cats stimulated electrically were able to replace completely the potassium lost in the saliva during stimulation. Glands stimulated with pilocarpine lost this restorative power as the output increased. It is suggested that pilocarpine has some specific effect on the intake of potassium by the gland.

Our thanks are due to Mrs. Doris Cobb Marsh for some of the potassium analyses used in this paper.

## REFERENCES

ANREP, G. V. J. Physiol. **54:** 319, 1921.
BROWN, J. B. AND N. J. KLOTZ. J. Dent. Res. **16:** 19, 1937.
FENN, W. O. AND D. M. COBB. This Journal **115:** 345, 1936.
GREGERSEN, M. I. AND E. N. INGALLS. This Journal **98:** 44, 1931.
HENDERSON, Y. This Journal **3:** 19, 1900.
PAVLOV, S. P. Centralb. f. Physiol. **2:** 137, 1888.
WACH, E. C. J. Dent. Res. **15:** 265, 1936.

# THE EFFECTS OF THE INGESTION OF GLUCOSE AND OF FRUCTOSE ON THE RATE OF EXCRETION OF URINE AND VARIOUS CONSTITUENTS

GEORGE BACHMANN, JOHN HALDI, CHARLES ENSOR AND WINFREY WYNN

*From the T. T. Fishburne Laboratory of Physiology, Emory University, Emory University, Georgia*

Received for publication June 23, 1938

Although a study of the urine as affected by the ingestion of glucose and of fructose might conceivably contribute to a further understanding of the metabolism of these sugars, the literature contains relatively few studies of this nature with glucose and none with fructose. Both diuresis and retention of water have been observed after the administration of glucose. Sugars administered subcutaneously, intravenously or by mouth have been found to induce diuresis (1). Similar observations were made by Bruger and Mosenthal (2) in 3 of 6 subjects following the ingestion of 100 grams of glucose. Byrom (3) found that diabetics, when given sufficient insulin to prevent glycosuria and ketosis and restricted to a diet of pure glucose, lost several pounds of body weight which he accounted for by a depletion of extracellular and intracellular water. Since the same results were obtained on a normal subject who consumed 600 grams of glucose a day without developing glycosuria, the author concluded that the loss of body fluid was a specific effect of glucose. McClendon (4) on the other hand found a marked retention of water when 400 grams glucose were taken in 2400 grams of water.

Nitrogen excretion was determined by Katayama (5) on five pathological subjects following the ingestion of 1.75 gram of glucose per kilogram of body weight. In some instances he found an increase and in others a decrease in the total urinary nitrogen. In three similar experiments on normal subjects there was an increase in the inorganic phosphorus of the urine, and in one experiment a decrease. These results, however, are open to question because of the paucity of data and the lack of controls. Retention of phosphorus has been observed in experiments with insulin during the time in which it was actively influencing metabolism (6–8). This has been interpreted as indicating a shift of phosphate from the blood into the tissues during the metabolism of carbohydrate. While these experiments may throw light on the mechanism of carbohydrate metab-

olism, they do not necessarily indicate that phosphate is retained when glucose is ingested under normal conditions. In this connection it should be noted that in glucose tolerance experiments (8) in which there was a marked rise in blood sugar, excretion of phosphate in some experiments showed an increase and in others a decrease, whereas it was uniformly diminished when insulin was administered before taking the glucose.

In the experiments here reported, we have studied the rate of excretion of the urine and its various constituents as affected by 50 grams of glucose or of fructose. This amount was chosen as it is known to produce a pronounced rise in the respiratory quotient and does not lead to an intestinal disturbance. Since wide variations occurred in the basal output of the urinary constituents from day to day and in the hourly excretion when water alone was taken, it was necessary to have a sufficient number of experiments for a statistical analysis.

METHOD. Five normal male adults served as subjects. Seventy-five experiments were done, 25 with water (controls) and the same number with glucose and with fructose. The same number of experiments was carried out with water and the sugars on each of the 5 subjects. On the morning of the experiment the bladder was emptied upon arising (7 a. m.) and 500 cc. of water ingested. About 30 or 45 minutes after arising the subject came to the laboratory without breakfast, reclined on a couch for 30 minutes and again emptied the bladder, the urine being discarded. He then reclined for 45 minutes and at the end of this time the basal collection of urine was obtained. Immediately afterward he drank 500 cc. water (control experiments) or 50 grams of glucose or of fructose dissolved in this volume of water and then reclined for one and three-quarter hours, after which time the bladder was emptied for the post-ingestion collection. This time was believed ample for any changes in the urinary constituents that might be induced by the sugars to become manifest, since the respiratory quotient reaches its maximum height within 45 minutes after the ingestion of 50 grams of glucose or of fructose and returns near the base line within one and three-quarter hours (9, 10).

The titratable acidity of the urine was determined by the Folin (11) method, the urine being shaken for about 5 minutes after the addition of potassium oxalate and then titrated with 0.01 N NaOH; total nitrogen by the Kjeldahl method essentially as described by Hawk and Bergeim (12) with methyl red-methylene blue as the indicator in the titration; ammonia by the permutit method of Folin and Bell (13); phosphorus by the method of Youngburg and Youngburg (14); preformed creatinine by the Folin method (15) with 10 cc. of urine in a 500 cc. flask (12); creatine as creatinine by the same colorimetric method after boiling the acidified urine with granulated lead on a hot plate (15); lactic acid by the Friedemann, Cotonio and Shaffer method (16). Interfering substances were removed with

copper-lime (17). Before beginning the oxidation with permanganate, the filtrates were boiled slowly and aerated for approximately 5 minutes through Erlenmeyer flasks containing water. The flasks were then washed, bisulphite introduced, the flasks replaced and the filtrate oxidized.

TABLE 1

*Average hourly excretion of the urine and its various constituents before and after the ingestion of water (control experiments), glucose, and fructose*

| | Period of excretion | Control experiments (water) | | Glucose experiments | | | Fructose experiments | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Average* excretion | σ | Average* excretion | σ | Diff.† / σ Diff. | Average* excretion | σ | Diff.† / σ Diff. |
| Volume (cc.) | Basal | 389 | ±121 | 346 | ±101 | | 328 | ±96 | |
| | P-I‡ | 328 | ±46 | 271 | ±53 | 4.1 | 213 | ±55 | 8.0 |
| Titratable acidity (cc. 0.1 N acid) | Basal | 7.3 | ±3 | 8.1 | ±3 | | 8.7 | ±4 | |
| | P-I | 5.6 | ±2 | 8.1 | ±3 | 3.4 | 10.6 | ±3 | 6.8 |
| Ammonia (mgm.) | Basal | 18 | ±7 | 22 | ±11 | | 20 | ±11 | |
| | P-I | 12 | ±5 | 20 | ±9 | 3.9 | 20 | ±7 | 4.7 |
| Lactic acid (mgm.) | Basal | 9 | ±3 | 8 | ±2 | | 8 | ±2 | |
| | P-I | 7 | ±2 | 7 | ±2 | 0 | 11 | ±4 | 4.5 |
| Total P (mgm.) | Basal | 26 | ±8 | 26 | ±8 | | 26 | ±7 | |
| | P-I | 23 | ±8 | 23 | ±8 | 0 | 26 | ±7 | 1.4 |
| Inorganic P (mgm.) | Basal | 23 | ±7 | 24 | ±8 | | 24 | ±7 | |
| | P-I | 21 | ±8 | 21 | ±8 | 0 | 24 | ±7 | 1.1 |
| Organic P (mgm.) | Basal | 3 | ±1 | 2 | ±1 | | 2 | ±1 | |
| | P-I | 2 | ±1 | 2 | ±1 | 0 | 2 | ±1 | 0 |
| Creatinine (mgm.) | Basal | 67 | ±11 | 68 | ±11 | | 69 | ±13 | |
| | P-I | 60 | ±10 | 65 | ±9 | 1.9 | 67 | ±10 | 2.0 |
| Nitrogen (mgm.) | Basal | 558 | ±86 | 576 | ±108 | | 583 | ±106 | |
| | P-I | 481 | ±69 | 519 | ±63 | 1.7 | 508 | ±76 | 1.3 |

* Twenty-five experiments.

† Ratio of the difference between the post-ingestion rate of excretion in the sugar and control experiments and the sigma of the difference.

‡ Post-ingestion.

Results. The average hourly excretion of the urine and its various constituents for the three groups of experiments is given in table 1. The figures on creatine excretion are omitted, for as will be shown later, the

average values do not bring out the full significance of the observations. The significance of the difference in the post-ingestion rate of excretion between the control and glucose or fructose experiments was determined by the value of the ratio, $\frac{M_1 - M_2}{\sigma(M_1 - M_2)}$ where $M_1$ and $M_2$ were the first and second mean, respectively, and $\sigma\ (M_1 - M_2)$ the unreliability of the difference between the means; this in turn was calculated from the formula, $\sigma\ (M_1 - M_2) = \sqrt{(\sigma M_1)^2 + (\sigma M_2)^2}$, the sigma of the mean $(\sigma M)$ being obtained by dividing the standard deviation $(\sigma)$ of the variate by the square root of the number of observations $\left(\frac{\sigma}{\sqrt{N}}\right)$. The ratios of the difference of the means for the post-ingestion excretion in the glucose and fructose experiments with reference to the controls are given in columns 7 and 10, respectively. A ratio of 3 has been taken to indicate a significant difference between the means, since in such a case the probabilities against the difference being due to a chance occurrence are 369 to 1. A ratio less than 2 is regarded as not showing a definite significance in the difference of the means. By this criterion the differences between the basal excretion of the water, glucose and fructose experiments were of no significance. This would indicate that the experiments were satisfactorily controlled.

The rate of excretion of total, inorganic and organic phosphorus and of creatinine and nitrogen was not affected by the ingestion of the sugars.

The volume excretion was significantly lower in the glucose and fructose experiments than in the controls. It was also lower in the fructose than in the glucose experiments. The difference between their rate of excretion was 3.8 times its sigma and was therefore significant.

The titratable acidity of the urine was significantly increased by taking the sugars, and to a greater extent by fructose than by glucose. The difference between the glucose and fructose experiments was significant as it was 2.9 times its sigma. The ammonia content of the urine was also increased, but there was no significant difference in the increase produced by the two sugars.

The lactic acid excretion was the same in the glucose and control but significantly higher in the fructose experiments. The actual increase produced by the ingestion of fructose was small, but is nevertheless of interest in view of the rise in blood lactic acid observed after the ingestion of fructose (18, 19).

The observations on creatine excretion, as stated above, are not recorded in the table for the reason that the averages would not reveal the full significance of the results. In a previous study (20) creatine was frequently found in the urine after the ingestion of glucose and of fructose. In the present study it was excreted after taking glucose in 5 out of 10 experi-

ments on the two subjects used in the former investigation, but only in 1 out of 15 experiments on the other 3 subjects. The average of the rate of excretion of the 25 experiments, which was only 0.4 mgm. per hour, would therefore be misleading as in the six experiments in which creatine was present, the rate of excretion varied from 1 to 2 mgm. per hour. After the ingestion of fructose, creatine was excreted in 23 out of 25 experiments, the excretion ranging from 1 to 4.5 mgm. per hour with an average of 1.9 mgm. In none of the experiments was creatine found in the basal collection. These results, therefore, confirm the previous findings of a higher average excretion of creatine induced by fructose than by glucose. They show also that creatine excretion is induced more frequently by fructose than by glucose and that some subjects do not excrete creatine after taking glucose, whereas all those we have studied excreted it in almost every instance after fructose. In these, as in the experiments previously reported, creatine excretion was not accompanied by a corresponding diminution in the creatinine excretion.

## SUMMARY

The rate of excretion of the urine and various constituents has been studied for a period of one and three-quarter hours following the ingestion of water (control experiments), 50 grams glucose and 50 grams fructose.

The volume of urine excreted was reduced by the sugars and to a greater extent by fructose than by glucose.

The titratable acidity of the urine was significantly higher after taking the sugars. The increase was larger in the fructose than in the glucose experiments.

The ammonia excretion was also increased by the sugars but there was no significant difference in the effect produced by the two sugars.

There was a small, but nevertheless significant, rise in the rate of lactic acid excretion after fructose but not after glucose.

Creatine appeared in the urine in 6 out of 25 experiments with glucose and in 23 out of 25 experiments with fructose.

The rate of excretion of phosphorus (total, inorganic and organic), creatinine and nitrogen was not affected by the ingestion of the sugars.

## REFERENCES

(1) Szelöczey, J. Ztschr. f. d. ges. exper. Med. **70:** 294, 1930.
(2) Bruger, M. and H. O. Mosenthal. Arch. Int. Med. **50:** 358, 1932.
(3) Byrom, F. B. Clinical Science **1:** 245, 1933-34.
(4) McClendon, J. F. This Journal **98:** 216, 1931.
(5) Katayama, I. J. Lab. and Clin. Med. **11:** 1024, 1925-26.
(6) Harrop, G. A. and E. M. Benedict. J. Biol. Chem. **59:** 683, 1924.
(7) Perlzweig, W. A., E. Latham and C. S. Keefer. Proc. Soc. Exper. Biol. and Med. **21:** 33, 1923-24.
(8) Blatherwick, N. R., M. Bell and E. Hill. J. Biol. Chem. **61:** 241, 1924.

(9) Carpenter, T. M. and E. L. Fox. Arbeitsphysiol. **4:** 532, 1931.
(10) Bachmann, G. and J. Haldi. J. Nutrition **13:** 157, 1937.
(11) Folin, O. This Journal **9:** 265, 1903.
(12) Hawk, P. B. and O. Bergeim. Practical physiological chemistry. Philadelphia, P. Blakiston's Son & Co., 1931.
(13) Folin, O. and R. D. Bell. J. Biol. Chem. **29:** 329, 1917.
(14) Youngburg, G. E. and M. V. Youngburg. J. Lab. and Clin. Med. **16:** 158, 1930–31.
(15) Folin, O. J. Biol. Chem. **17:** 469, 1914.
(16) Friedemann, T. E., M. Cotonio and P. A. Shaffer. J. Biol. Chem. **73:** 335, 1927.
(17) Van Slyke, D. D. Quantitative clinical chemistry. Vol. II, Methods. Baltimore, The Williams & Wilkins Co., 1932.
(18) Campbell, W. R. and E. J. Maltby. J. Clin. Investigation **6:** 303, 1928–29.
(19) Haldi, J. and G. Bachmann. J. Nutrition **14:** 287, 1937.
(20) Haldi, J. and G. Bachmann. This Journal **115:** 364, 1936.

# THE EFFECT OF COCAINE AND OF ERGOTAMINE ON THE ACTION OF THE RENAL PRESSOR SUBSTANCE[1]

JOHN R. WILLIAMS, JR.

*From the Department of Medicine, Vanderbilt University, Nashville, Tennessee*

Received for publication June 11, 1938

In a previous report (1) it was shown that, under certain conditions, an agent having some of the chemical and pharmacologic properties of adrenalin may be obtained by perfusing the kidney. Pending further knowledge of the chemical nature of this substance the name "perfusin" was applied to it. Since this agent could be more readily recovered from kidneys which had been removed from the body for one or more hours than from freshly removed kidneys it seemed that it might possibly be derived from the disintegration of some more complex compound. The possibility that perfusin was simply a non-specific product of protein autolysis seemed unlikely because *a*, its pharmacologic properties were quite different from those of tyramine, the pressor substance usually derived from protein disintegration (2), and *b*, it was not obtained from organs other than the kidney. These facts led to the idea that perfusin might be specifically derived from renin (the renal pressor substance having the properties of a protein which was described by Tigerstedt and Bergmann (3)). According to this concept the prolonged rise in blood pressure produced by renin could be ascribed to the slow release of the adrenalin-like substance. If such were the case, one would expect that the action of renin might be enhanced or inhibited by substances having a similar effect on the action of perfusin. Since the previous study (1) showed that the pressor effect of perfusin was, like that of adrenalin, increased by cocaine and diminished by ergotamine, observations were made concerning the relation of these agents to the action of renin.

Rats weighing 140 to 240 grams and anesthetized by the intraperitoneal injection of sodium pentobarbital were used as test objects. A cannula was inserted into the abdominal aorta just proximal to its bifurcation and was connected to a mercury manometer, heparin being used to prevent clotting. Injections were made into the aorta through a three-way tap.

Renin was prepared by treating minced renal cortex of hogs with nine volumes of alcohol, according to the technique described by Grollman (4). Three series of animals were studied, each including four to five rats. One group received renin

[1] Aided by a grant from the Josiah Macy, Jr. Foundation.

alone. Another group had renin after 0.05 mgm. of ergotamine, and the third group received renin after five milligrams of cocaine. Doses of renin were given according to the body surface of the animals and were calculated by the formula:

$$\frac{(\text{Body weight in grams})^{2/3}}{100} \times 2 = \text{cc. to be injected}$$

Following the injections of renin the blood pressures were followed for fifteen minutes.

The results of the experiments were striking (fig. 1) and indicated clearly that ergotamine inhibits and cocaine enhances the pressor action

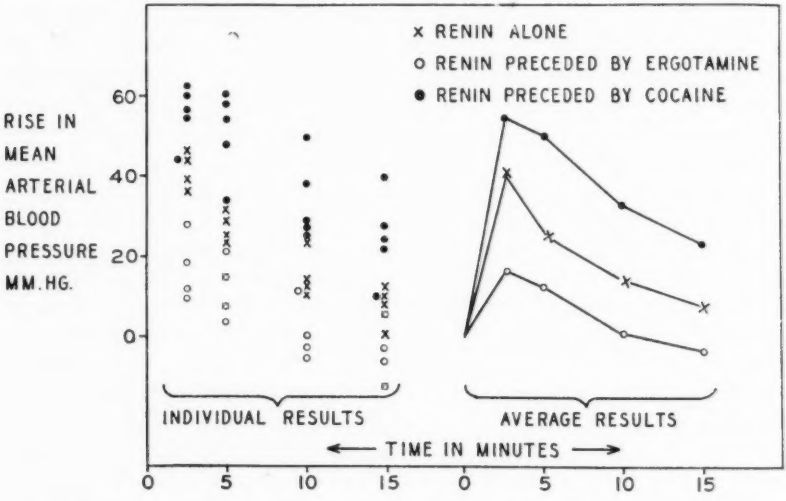



Fig. 1. Results of single experiments are plotted on the left and average results are shown at the right. For the sake of simplicity the maximum rise following each injection is recorded as if it had invariably occurred two and one half minutes later. Actually the maximum response appeared from one to three minutes in different animals.

The figure shows clearly that the animals receiving ergotamine previously had less rise in blood pressure when given renin than did those receiving renin only. The previous administration of cocaine had the reverse effect.

of renin. Since the effects of both adrenalin and perfusin are similarly modified by the drugs, the findings offer support to the assumption that the prolonged rise in blood pressure produced by renin may be due to the gradual liberation of an adrenalin-like substance.[2] However, this assump-

[2] Another possible explanation of the findings, i.e., that the injection of renin causes release of adrenalin from the suprarenal glands, is untenable in view of the fact that removal of the adrenal medulla does not alter the pressor effect of renin (5).

tion, although in accord with the available evidence, can not be regarded as proven at the present time.

SUMMARY

The rise in blood pressure produced in white rats by the injection of the renal pressor substance (renin) is enhanced by cocaine and inhibited by ergotamine.

REFERENCES

(1) Williams, J. R. and E. B. Grossman. This Journal. In press.
(2) Williams, J. R., Jr., T. R. Harrison and M. F. Mason. Am. J. Med. Sci. **195:** 339, 1938.
(3) Tigerstedt, R. and P. G. Bergmann. Skand. Arch. Physiol. **8:** 223, 1898.
(4) Grossman, E. B. In press.
(5) Williams, J. R., Jr., J. T. Diaz, J. C. Burch and T. R. Harrison. To be published.

# FURTHER STUDIES IN THE MECHANISM OF VASCULAR HYPERTENSION FOLLOWING THE INTRACISTERNAL INJECTION OF KAOLIN IN THE RAT

J. Q. GRIFFITH, JR.[1] AND E. ROBERTS

With the technical assistance of R. CAMPBELL

*From the Robinette Foundation and the Medical Clinic of the Hospital of the University of Pennsylvania*

Received for publication June 21, 1938

Heller (1) and others have described the occurrence of vascular hypertension in the dog following the intracisternal injection of colloidal kaolin. This was attributed to an increased intracranial pressure. Griffith, Jeffers and Lindauer (2) produced a similar syndrome in white rats and, using thorotrast, demonstrated a block in the perineural absorption from the cerebrospinal space. Later, they (3) measured the cerebrospinal fluid pressure, and found it to be about 260 mm. of water, as compared with a normal pressure not exceeding 100.

The present studies, some of which were suggested by results obtained during the course of the work, include: *a*, onset, frequency and duration of vascular hypertension following the intracisternal injection of colloidal kaolin; comparison of indirect "systolic" and direct "mean" pressures; *b*, "capillary" or minute vessel pressure and its variation in the area of a flare; *c*, blood volume; *d*, water content of the brain; *e*, absorption from the cerebrospinal space into the blood stream; *f*, effect of complete adrenalectomy upon the hypertension; *g*, effect of kaolin upon the patency of the perivascular spaces of the cerebral cortex; *h*, comparison of the thickness of the gray matter of the cerebral cortex in animals with longstanding hypertension, in normal animals, and in animals with implanted cerebellar tumors; *i*, effect of carotid sinus stimulation upon the blood pressure of the kaolin hypertensive animal.

*a. Incidence of hypertension. Comparison of "systolic" and "mean" pressures.* In the past three years over a thousand rats have been given kaolin by cisternal injection. It is probable that every rat that received enough kaolin into the cistern developed the syndrome. However, when one cisternal puncture was made to remove fluid and a second to inject kaolin, it was not unusual to secure only 1 or 2 hypertensive animals out of a dozen injected. For the past 6 months a micropipette has been used

[1] Atwater Kent Fellow in Medicine.

which permits withdrawal of fluid into one side-arm and injection of kaolin suspension through another without removing the needle. With this technique the percentage of successful injections, as evidenced by the occurrence of vascular hypertension, is much higher. Preliminary work showed that hypertension did not occur before the 5th day after the injection (2). This was interpreted as meaning either that the essential pathology necessary for the hypertension did not occur before the 5th day, or that the animal was too sick following the injection to maintain a hypertension prior to this time. It had also been previously noted (2) that the leucocytosis in the spinal fluid subsided about the 5th day. Animals which were hypertensive on the 5th day were marked and placed in special cages. The remainder had their blood pressures taken again on the 12th day, and those that were not high were discarded or re-injected. Hypertensive animals had blood pressures taken at approximately weekly intervals. Most animals were sacrificed for acute experiments prior to the 6th week, but a few lived 3 months. We have kept accurate records of the fate of the last 53 animals injected.

Blood pressure measurements were made by the indirect method of Griffith (4) under ether anesthesia. This reading has been shown to correspond to systolic pressure. Diastolic pressure cannot be obtained. Mean pressure was obtained in 4 animals by inserting a needle into the abdominal aorta. This needle was connected through tubing filled with water to a mercury manometer with such inertia that there was no pulsation. The reading obtained was taken as mean pressure. In performing the test a little blood was lost into the manometer system, corresponding to 0.33 cc. per 100 mm. of mercury. Moreover, an abdominal incision was required. Both these factors tend to lower the mean pressure reading. Three normal animals were treated similarly.

Of the last 53 animals injected with kaolin, 43 developed a vascular hypertension and 10 did not. Of the 43, 29 were hypertensive on the 5th day, while 14 more developed a hypertension by the 12th day. In 5 animals pressures which were high on the 5th day had fallen to normal by the 12th day, and stayed down. Of the 38 that were hypertensive on the 12th day, 8 had normal pressures by the 6th week and 24 had been sacrificed in acute experiments. Six animals survived and remained hypertensive for from $1\frac{1}{2}$ to $2\frac{1}{2}$ months.

Table 1 shows the comparison of systolic and mean blood pressure in 3 normal animals and in 4 animals with hypertension. The mean pressure is elevated, though not greatly. The systolic pressures as obtained were lower than those recorded a few days earlier, probably because of the trauma of opening the abdomen.

*b. "Capillary" or minute vessel pressure.* "Capillary" or minute vessel pressure was obtained as follows: The dorsum of the foot was shaved

several days in advance. Then, under ether anesthesia, the rat was placed upon a mechanical stage with the foot supported by plasticine. A Danzer-Hooker apparatus was used, with two modifications: The compressing chamber was attached to a rod held in a micromanipulator; the compressing chamber was filled with mineral oil, which aids visibility. The compressing chamber is moved over the foot and lowered so that contact is made at one point. At this point, through the low power of the microscope using the Ultrapak attachment, "capillary" flow is visualized. The compressing chamber is then lowered until it is certain that good contact has been secured. Usually this slows the flow slightly. Then the pressure in the compressing chamber is raised until flow stops, at which point the "capillary" disappears. This pressure is then recorded. The compression chamber is then raised and the foot touched with a red hot wire about 5 mm. from the point of observation so that this point is included in the flare area. In 4 to 5 minutes from the time of the burn a

TABLE 1

*Table showing relation between indirectly obtained "systolic" pressure and directly obtained "mean" pressure in normal animals and in animals made hypertensive by the intracisternal injection of kaolin*

| | ANIMAL NUMBER | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5* | 6* | 7* | 8* |
| Blood pressure: | | | | | | | | |
| Direct | 71 | 60 | 70 | 74 | 120 | 100 | 108 | 105 |
| Indirect | 130 | 90 | 100 | 120 | 160 | 144 | 190 | 165 |

* Animal hypertensive following the intracisternal injection of kaolin.

second "capillary" pressure reading is obtained on the same vessels. As these vessels have not been identified histologically, the term minute vessel is probably preferable to "capillary." Although numerous normal control experiments have been performed, only 3 representative ones are recorded as comparison with the 5 hypertensive animals.

Table 2 shows the minute vessel pressures in 4 hypertensive and 3 normal animals. The hypertensive animals all have an initially elevated minute vessel pressure which falls in the flare area of a burn, the reverse of the change that occurs in the normal animals.

*c. Blood volume.* Blood volume measurements were made in 11 hypertensive animals under nembutal anesthesia by the method of Griffith and Campbell (5). Blood volumes obtained, in terms of cubic centimeters per 100 gram body weight, are 4.2, 4.5, 4.1, 5.1, 5.0, 5.5, 4.7, 5.0, 4.1, 5.5, 5.3, in animals with blood pressures of 200, 220, 160, 170, 175, 154, 220, 185, 155, 200, 180 mm. of mercury. Thus the range is 4.1 to 5.5,

averaging 4.8. The normal range is 4.0 to 5.3. There is no evidence of alteration of blood volume during kaolin hypertension.

*d. Water content of the brain.* Water content of the brain was measured in 6 animals with hypertension, by heating the brain to 110°C. for 72 hours and measuring the loss of weight. In addition two hypertensive animals were given 15 cc. of water per 100 grams body weight subcutaneously, killed in 24 hours, and the water content of the brain determined.

The water content of the brain was 77.6 per cent, 79.0 per cent, 78.9 per cent, 78.0 per cent, 78.2 per cent, and 77.9 per cent, in animals with blood pressures of 190, 165, 160, 200, 210, 150 mm. of mercury. Thus the range is from 77.6 to 79.0, averaging 78.2. In two animals with blood pressures of 170 and 185 that were given water subcutaneously, water content of the brain was 78.0 and 78.4. The normal average previously established (3) is 78.0, with a range of 77° to 79.2.

TABLE 2

*Table showing minute vessel pressure in normal animals and in animals made hypertensive by the intracisternal injection of kaolin. Change in minute vessel pressure in the flare area of a burn*

| | ANIMAL NUMBER | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1* | 2* | 3* | 4 | 5 | 6 | 7 | 8 |
| Minute vessel pressure before burn | 16 | 18 | 14 | 35 | 35 | 32 | 40 | 52 |
| Minute vessel pressure after burn | 28 | 40 | 18 | 26 | 10 | 19 | 22 | 24 |
| Blood pressure (systolic) | | | | 210 | 155 | 230 | 190 | 220 |

* Normal animal.

*e. Absorption into the blood stream.* Absorption from the cerebrospinal space into the blood stream was measured by injecting phenolsulphonphthalein into the cistern and obtaining samples of blood from the tail veins at approximately 30 second intervals. Five hypertensive and 4 normal animals were given 0.03 cc. of 0.6 per cent phenolsulphonphthalein. Six hypertensive and 5 normal animals were given 0.03 cc. of 1.5 per cent phenolsulphonphthalein after preliminary ligation of the renal pedicles. As the cisternal injection requires 20 to 30 seconds and the animal had to be removed from a stand, it was seldom that a tail vein could be punctured and the collection begun in less than 90 seconds after the beginning of the injection. A stop watch was used for timing. Blood samples were collected in narrow tubes filmed with oxalate. After centrifuging the clear serum was transferred to another tube containing alkali. If dye was present, a pink color developed. If the original serum was pink indicating hemolysis, it was discarded. In 4 animals 0.03 cc. of 1.5 per cent phenol-

sulphonphthalein was injected into the tissues of the back of the neck outside the cistern, and the balance of the procedure carried out as just described.

In the dye absorption experiments, dye appeared in the blood at approximately the same time in the normal and in the hypertensive animals, as shown in figure 1. In the animals given the weaker dilution of the dye the color was only a faint tint, and was absent, usually, in a sample taken a minute later. These experiments were done first and the times recorded. Because of the faintness of the color, later experiments were performed with renal pedicles ligated and using a stronger solution of the dye. In such animals the color is quite marked and remains for at least 15 minutes, which is the longest time any animal was followed. Therefore in this group only the first specimen in which the dye appeared is recorded. None of the 4

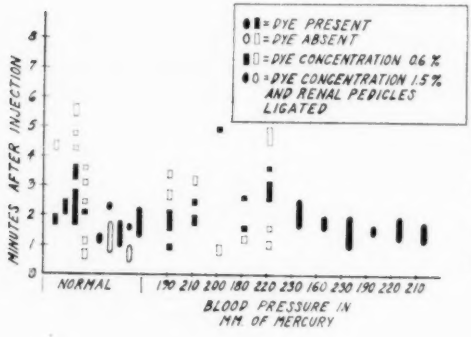



Fig. 1

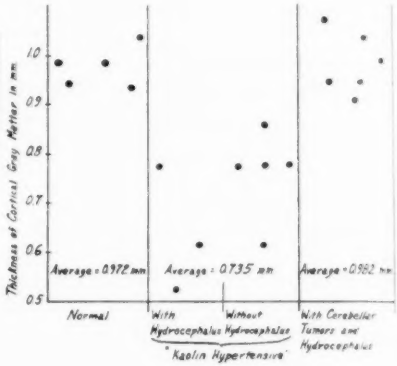



Fig. 2

animals given dye outside the cistern showed any color in the blood, after following a similar procedure. This was accepted as evidence that only dye entering the cerebrospinal space appeared in the blood, under the conditions of the experiment.

*f. Effect of adrenalectomy.* Twenty-five rats had bilateral adrenalectomies performed in two stages a week apart. They were maintained on physiologic saline as drinking fluid, but otherwise had no substitution therapy. Five days after the second adrenalectomy the survivors received an intracisternal injection of kaolin. Blood pressure measurements were made on the 5th and 12th days following the injection. Water was then substituted for the physiologic saline, and the survivors killed 2 weeks later. All dead animals, whether dying spontaneously or killed, were carefully posted and suspicious tissue stained specifically.

Eight animals with complete adrenalectomies developed hypertensions

of 180, 155, 200, 170, 160, 170, 195, 185 mm. of mercury. Five of these died in apparent adrenal deficiency when water was substituted for physiologic saline, and 3 were killed. At autopsy none showed any adrenal tissue remaining. Two animals which had a little residuum of cortical tissue are excluded from this series. It appears, therefore, that rats can develop a vascular hypertension after the intracisternal injection of kaolin even after complete adrenalectomy.

*g. Patency of the perivascular cortical spaces.* Fourteen normal and 10 hypertensive animals were given 15 cc. of water subcutaneously and killed 24 hours later. Formalin was injected into the carotid artery, the brain fixed in situ and subsequently removed and sectioned. The perivascular spaces leading to the surface of the cortex were carefully examined. All 14 normal animals given 15 cc. of water subcutaneously and killed 24 hours later showed marked widening of the perivascular spaces near the surface of the cortex. In 9 out of 10 hypertensive animals treated similarly these perivascular spaces were normal. However, some of the more deeply situated perivascular spaces were moderately dilated. It is probable that kaolin, entering the perivascular spaces from the cerebrospinal space, had caused an inflammatory reaction which subsequently tended to block the perivascular spaces.

*h. Thickness of the gray matter of the cerebral cortex.* In our collection of brains preserved in formalin it was possible to obtain the brains of 6 rats dying from implanted cerebellar tumors, of 8 rats that had received kaolin and had maintained a vascular hypertension for 6 weeks to 3 months. These brains were of the same age and had been preserved in exactly the same way, so that it was felt that measurements in the two groups could be compared. To these were added the brains of 5 recently killed normal animals. Sections were made of the superior surface of the brain 6 mm. in front of the cerebellum and 3 to 6 mm. lateral from the midline. The sections were stained with Weigert's stain and the thickness of the gray matter of the cortex measured with an ocular micrometer.

The thickness of the gray matter of the cortex preserved in formalin for some time from 6 animals with cerebellar tumor and internal hydrocephalus was approximately the same as in 5 recently killed normal animals. On the other hand, the thickness of the gray matter is definitely less in 8 animals with longstanding hypertension following kaolin. This is shown in figure 2. Of the 8 kaolin animals, 3 had marked hydrocephalus and 5 had no or questionable hydrocephalus. The gray matter was a little thicker in the animals without hydrocephalus than in those with hydrocephalus, though still definitely below the normal range. While the explanation for this is uncertain, it is suggested that the block of the perivascular spaces may be of sufficient degree to lead to atrophy on a nutritional basis.

*i. Effect of carotid sinus stimulation.* Stimulation of the carotid sinus

region was performed in 5 animals with marked hypertension. The right fork was exposed in 3 animals and the left in 2. Ether anesthesia was used. Slow Faradic stimulation was employed of a strength which had been shown to cause a fall in blood pressure when applied to the carotid bifurcation in normal animals. Stimulation lasted approximately 30 seconds. Blood pressure measurements were made as rapidly as possible, i.e., one every 30 to 40 seconds.

Blood pressure readings obtained within a minute of the beginning of carotid sinus stimulation showed a sharp drop in pressure, which rose again to its original level in 1–6 minutes after the stimulation was discontinued. As the method for measuring blood pressure requires an interval of at least 30 seconds, it is possible that the extreme low point of the drop may have been missed, at least in certain cases. Blood pressure measurements as obtained showed drops from 300 to 120, 190 to 125, 228 to 80, 290 to 120, and 300 to 170.

Discussion and summary. When kaolin is injected into the cerebrospinal space the following train of events is thought to occur:

1. An irritative mechanical meningitis with leucocytosis in the spinal fluid, subsiding by the 4th to 5th day.

2. Kaolin entering the perineural sheaths sets up an inflammatory reaction which tends to obstruct these spaces. This reaction is at its height in 5 to 12 days but tends to subside later. In healing it may or may not result in permanent blockage, depending perhaps upon the amount of kaolin originally present. Recovery tends to occur in 1 to 3 months, or even earlier.

3. Probably kaolin, in varying amounts, enters the perivascular spaces leading to the subarachnoid space, and tends to block them just as it does the perineural spaces. This leads to interference with elimination of the normal intercellular fluid of the brain, which probably has to pass mainly to the ventricles instead of following its usual route to the subarachnoid space. This change is made more apparent when the intercellular fluid is increased by loading the animal with excess fluid. This may be a factor in the production of the hydrocephalus, which is certainly of the communicating type, because *a*, the subarachnoid pressure is increased, and *b*, thorotrast injected intracisternally both enters the ventricle and outlines the subarachnoid space.

4. In spite of the probable difficulty in eliminating intercellular fluid, there is no change in the percentile water content of the brain.

5. The gray matter of the cortex is thinned out of proportion to the degree of ventricular dilatation. In fact, the cerebellar tumor animals had more hydrocephalus than did the kaolin animals. This finding is consistent with the suggestion of impairment in elimination of inter-

cellular fluid, the cortex being most disadvantageously situated if the major part of the fluid must pass to the ventricle.

6. There is no impairment of absorption from the cerebrospinal space into the blood stream. The increase in cerebrospinal fluid pressure would therefore appear to be the result of the obstruction of perineural absorption to the systemic lymphatic system.

7. Blood volume is normal. Therefore an increased blood volume is not the mechanism of the production of the hypertension.

8. The hypertension is not dependent upon an increased secretion of adrenalin, and can occur in the absence of both adrenals. This was demonstrated also in the case of dogs by Jeffers, Lindauer and Lukens (6).

9. The increased capillary pressure is consistent with the suggestion that the hypertension is the result of increased peripheral vascular tonus, including the capillaries. The fall of pressure in the flare area also suggests that the capillaries themselves had been sharing in the increased tonicity. An alternative suggestion is that in the rat the pressure reading by this method may really be of vessels of a higher order than capillaries, and might therefore be higher than capillary pressure.

10. There is an elevation both of systolic and of mean blood pressure.

## REFERENCES

(1) Heller, H. Klin. Wchnschr. **13:** 241, 1934.

(2) Griffith, J. Q., Jr., W. A. Jeffers and M. A. Lindauer. This Journal **113:** 285, 1935.

(3) Griffith, J. Q., Jr., W. A. Jeffers and M. A. Lindauer. This Journal **118:** 1, 1937.

(4) Griffith, J. Q., Jr. Proc. Soc. Exper. Biol. and Med. **32:** 394, 1934.

(5) Griffith, J. Q., Jr. and R. Campbell. Proc. Soc. Exper. Biol. and Med. **36:** 38, 1937.

(6) Jeffers, W. A., M. A. Lindauer and F. D. W. Lukens. Proc. Soc. Exper. Biol. and Med. **37:** 260, 1937.

# THE INFLUENCE OF EXPERIMENTAL BILIARY OBSTRUCTION AND LIVER INJURY UPON THE TOTAL BILE ACID CONTENT AND PARTITION IN BLOOD AND URINE[1]

S. S. LICHTMAN

*From the Laboratories and the Medical Division, Service of Dr. George Baehr, of the Mount Sinai Hospital, New York, N. Y.*

Received for publication June 15, 1938

The influence of liver damage and biliary obstruction upon the bile acid metabolism of the liver has been reported by several observers. In some experiments analysis of fistula bile was made (1–4) and in others the cholic acid content of the blood and urine was determined (5). In the present study new methods were employed for the estimation of cholic acid and total bile acids which include desoxycholic acid (7). The estimation of total bile acids in the blood and urine permitted the comparison of the cholic and "non-cholic" acid fractions after liver injury or biliary obstruction.

PROCEDURE AND METHODS. The dogs employed were maintained for two weeks on a standard laboratory diet of meat and vegetables. Blood samples were drawn from the femoral artery in fasting dogs and the urine was obtained by catheter. Adequate urinary output was established by administering water by tube 1 to 2 hours preceding catheterization. Doses of 1, 2 or 3 cc. per kgm. of carbon tetrachloride were fed to 8 dogs. Double ligation and transection of the common bile duct was performed in 6 dogs, in 2 of which the gall bladder was also removed.

The author's hemolytic method (7) for estimating the total bile acid content of body fluids was employed. A slight modification of Josephson's procedure was used to estimate cholic acid in the blood. The alcoholic filtrate was evaporated to dryness before extraction with ethyl acetate. The residue was finally extracted with 60 per cent acetic acid following Schmidt's suggestion (8). The usual correction was made for the non-specific color formed by the action of sulfuric acid on the blood extract. An additional slight correction was made for the color formed by sulfuric acid and furfural. The preliminary treatment with barium and the final use of acetic acid reduced the pigment and turbidity factors to a minimum.

The Josephson procedure for the analysis of cholic acid in blood was adapted to urine analysis. Nine cubic centimeters of urine were precipi-

[1] I. J. Madorsky and Walter Mann rendered expert technical assistance.

tated with 7 volumes of absolute alcohol. The precipitate was separated, and the filtrate treated with 8 cc. of the barium solution. One and two-tenths cubic centimeters of 2N sulfuric acid was saved to remove the barium. In other respects the procedure for blood was followed, save that concentrations of 20, 25, or 30 mgm. cholic acid per 100 cc. were added directly to the residues of undiluted urines just before extraction with the acetic acid.[2] Experiments indicated that it was necessary to add these amounts to some urines to assure quantitative recovery of added cholic acid. Under these circumstances the color formed was found to nullify the interfering factors.

*Calculation of the total bile acid content and the cholic acid fraction.* The colorimetric value represents the amount of free cholic acid. This value divided by 7.6, the hemolytic equivalent factor for this bile acid, indicates the number of D.H.E. units of free cholic acid.[3] The difference between this concentration of D.H.E. units and the total concentration of D.H.E. units in milligrams per 100 cc. obtained directly by the hemolytic method represents the "non-cholic" acid fraction. When the concentration of this fraction is added to the colorimetric value, the absolute total value for bile acids in milligrams per 100 cc. is obtained.

*The total bile acid content and cholic acid fraction in normal blood and urine.* The total bile acid content of blood of fasting dogs varied between 3.0 and 14 mgm. and the urine between 2.5 and 22 mgm. per 100 cc. The cholic acid content of blood varied between 2 and 10 mgm. and the urine between 2.5 and 22 mgm. per 100 cc.

The cholic acid fraction was estimated in 17 dogs. In 24 analyses of blood in 15 dogs, cholic acid comprised 50 to 90 per cent of the total. In 22 analyses of the urine in 17 dogs cholic acid was found to be 90 to 100 per cent of the total. The percentage of cholic acid in the blood and urine of the same dog generally varied within 10 to 15 per cent on separate days.

*The effect of acute liver injury and complete biliary obstruction on the bile acid content of blood and urine estimated by the hemolytic method (D.H.E.).* Single doses of carbon tetrachloride which produce maximum liver injury histologically in 48 to 72 hours were used (9). In 8 dogs poisoned with this agent slight but definite increases occurred in the blood D.H.E. values. The 12 control values ranging between 0.5 and 6.0 mgm. per 100 cc.

[2] The dried residue obtained from an aliquot portion of 25 cc. of alcoholic filtrate represented the bile acid content of 3 cc. of original urine. After extraction with 3 cc. of 60 per cent acetic acid, 1 cc. was used for the estimation. Another cubic centimeter was used to determine the correction for the color formed by the action of sulfuric acid upon the extract of urine.

[3] The desoxycholate hemolytic equivalent (D.H.E.) is the quantitative unit of the hemolytic method expressed in milligrams per 100 cc. It represents the amount of bile salts of all types having the same hemolytic action as a definite amount of desoxycholate on a standardized erythrocyte suspension.

averaged 2.4 mgm. After poisoning, the averages rose to 3.6, 3.8, 3.9 and 3.6 mgm. per 100 cc. in 24, 48, 72 and 96 hours, respectively. The urinary values rose correspondingly over a period of 72 hours. The original values returned 7 days after injury.

TABLE 1

*The influence of acute liver injury induced by a single dose of carbon tetrachloride upon the bile acid content of the blood and urine of dogs estimated by the hemolytic method**

| DOG NUMBER | CONTROL BLOOD / URINE | DAYS AFTER CARBON TETRACHLORIDE BLOOD / URINE | | | | | | DOSE OF $CCl_4$ PER KGM. |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 6 | 7 | |
| | | | | | | | | cc. |
| 1 | 3.0 / 0.5 | 2.0 / 2.0 | 5.0 / 1.0 | 4.0 / 1.0 | 5.0 / 2.0 | | | 1 |
| 2 | 4.0 / 0.5 | 3.0 / 0.5 | 5.0 / 0.5 | 5.0 / 2.5 | | | 2.5 / 1.0 | 2 |
| 3 | 3.0 / 1.0 | 3.0 / 3.0 | 2.5 / 7.0 | 5.5 / 2.0 | 6.0 / 2.0 | 4.0 / 0.5 | | 3 |
| 4 | 0.5 / 1.0 | 1.0 / 0.5 | 2.0 / 2.5 | 3.5 / 4.0 | 5.0 / 1.0 | | 2.5 / 0.5 | 2 cc. ethyl alcohol 2 cc./kgm. |
| 5 | 2.0 / 0.5<br>3.0† / 2.5 | 4.0 / 2.0 | 5.0 / 6.0 | 5.5 / 1.0 | 3.5 / 1.0 | | 3.5 / 2.0 | 2 |
| 6 | 5.0 / 1.0<br>6.0† / 1.5 | 8.0 / 1.0 | 2.0 / 3.5 | 4.0 / 2.0 | 1.0 / 0.5 | | 0.5 / 0.5 | 2 |
| 7 | 1.0 / 0.5<br>0.5† / 0.5 | 3.5 / 0.5 | 3.0 / 0.5 | 2.0 / 0.5 | 0.5 / 0.5 | 2.0 / 0.5 | 1.0 / 0.5 | 3 |
| 8 | 0.5 / 0.5<br>0.5† / 0.5<br>2.0† / 0.5 | 3.5 / 2.0 | 3.0 / 3.5 | 0.5 / 4.5 | 1.0 / — | | 0.5 / 3.5 | 2 cc. ethyl alcohol 2 cc./kgm. |
| Average.... | 2.4 / 0.8 | 3.5 / 1.5 | 3.4 / 3.0 | 3.7 / 2.2 | 3.1 / 1.2 | 3.0 / 0.5 | 1.8 / 1.3 | |

* Results expressed as milligrams per 100 cc. desoxycholate hemolytic equivalent (D.H.E.).

† Duplicate control estimations made on successive days.

Common duct occlusion produced marked increases in D.H.E. values in the urine by the 6th day after ligation. With few exceptions this continued daily. The average control value of 1.5 mgm. per 100 cc. D.H.E. derived from 13 estimations in 6 dogs was raised to maximum values of

TABLE 2

*The influence of biliary obstruction upon the bile acid content of blood and urine*†

| DOG NUMBER | CONTROL BLOOD / URINE | DAYS AFTER COMMON DUCT LIGATION — BLOOD / URINE | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | 7 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 17 | 18 | 19 | 20 | 21 | 22 |
| 9* | 1.0 / 1.0 | 0.5 / 7.0 | 5.0 / 2.5 | | 5.0 / 6.0 | | 5.0 / 6.0 | | 3.5 / 8.5 | 2.0 / 12.5 | | | | | | |
| 10* | 2.0 / 1.0 | 3.0 / 7.5 | 3.5 / 5.0 | 3.5 / 5.0 | | | 2.5 / 3.0 | | 3.5 / 3.0 | | 2.5 / 3.0 | | 5.5 / 4.0 | | | |
| 11 | 4.0 / 0.5 | 4.0 / 2.0 | 2.5 / 7.5 | 3.5 / 5.0 | 3.5 / 5.0 | 3.5 / 8.5 | | 0.5 / 6.0 | | | | 4.5 / 15.5 | | 6.0 / 4.5 | | |
| 12 | 3.5 / 2.0 | 2.5 / 3.5 | 3.0 / 5.0 | 4.0 / 7.0 | | 5.5 / 7.0 | 2.0 / 9.0 | 2.5 / 10.5 | 3.5 / 9.0 | 4.0 / 9.0 | | 7.5 / 7.0 | 6.5 / 4.5 | | | |
| 13 | 4.0 / 1.0 / 6.0 / 2.0 | 5.5 / 3.0 | 4.0 / 3.5 | 3.0 / 6.5 | | 3.5 / 5.5 | 3.5 / 4.0 | 2.0 / 8.0 | 0.5 / 5.0 | | 1.0 / 3.0 | | 2.5 / 3.0 | | | 0.5 / 5.5 |
| 14 | 6.5 / 2.5 / 4.0 / 2.0 / 3.0 / 0.5 | 2.0 / 5.0 | 1.0 / 5.0 | 3.0 / 5.0 | | 3.5 / 7.0 | 1.0 / 5.5 | 1.0 / 5.0 | 4.0 / 3.0 | | | 3.5 / 3.0 | 2.5 / 7.0 | 2.5 / 3.0 | 3.0 / 3.5 | 5.0 / 6.0 |
| | 3.0 / 1.0 / 2.0 / 0.5 | | | | | | | | | | | | | | | |
| Average | 3.3 / 1.5 | 2.6 / 5.6 | 3.7 / 4.7 | 3.5 / 6.0 | | 4.0 / 7.0 | 2.8 / 5.5 | 1.5 / 7.4 | 3.0 / 5.7 | 3.0 / 10.8 | 1.7 / 3.0 | 5.1 / 8.8 | 4.2 / 4.6 | 4.2 / 4.6 | 3.0 / 3.5 | 2.7 / 5.8 |

* Double common duct ligation and transection and cholecystectomy.

† Results expressed as millimeters per 100 cc. of desoxycholate hemolytic equivalents (D.H.E.). Duplicate control estimations made on successive days.

10.8 and 8.8 mgm. per 100 cc. on the 15th and 18th days after ligation. Slight but definite rises in the average blood values occurred in the second week from an original value of 3.3 to 4.0 and somewhat higher, 5.1 mgm. per 100 cc. in the third week. In four dogs (nos. 10, 11, 12, 14) maximum

values in the blood were noted at the end of the experiment. The increased blood D.H.E. values following ligation were accompanied by increased urinary levels.

*The influence of acute liver damage and biliary obstruction on the total bile acid content and cholic acid fraction in blood and urine.* The effect of complete common duct obstruction was tested in two dogs. Figure 1 represents the results typical of common duct ligation. Save for two days, the blood and urinary totals increased. The maximum concentration in the blood reached 15 mgm. per 100 cc. on the 6th and 10th days after ligation. Urinary concentrations reached 40 mgm. There were daily fluctuations.

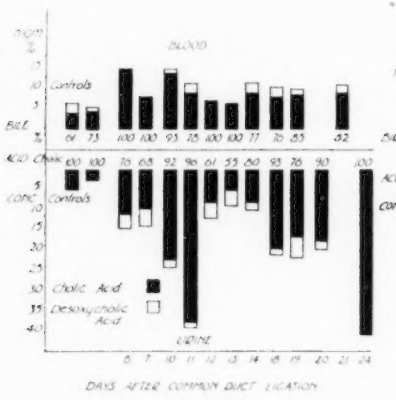



Fig. 1

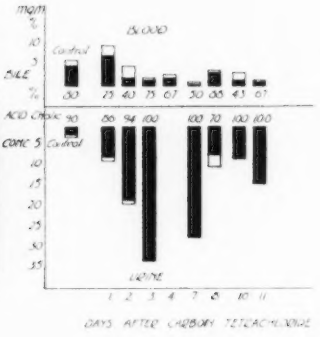



Fig. 2

**Fig. 1.** The effect of biliary obstruction on bile acid content and partition in the blood and urine (dog 14).

**Fig. 2.** The effect of liver damage on bile acid content and partition in the blood and urine (dog 8).

The elevation in the urine continued throughout the experiment. The increase in total concentration in the blood was due mainly to cholic acid, the original fraction mounting often to 100 per cent from the original level of 60 to 75 per cent. In the urine, the increase was also mainly in the form of cholic acid. On some days, however, the cholic acid fraction diminished from the original level of 100 per cent to as low as 55 per cent, e.g., 13 days after operation.

The effect of liver damage induced by carbon tetrachloride was tested in three dogs. Figure 2 represents a typical result. The total bile acids rose from 6.5 to 10 mgm. per 100 cc. of blood in the first 24 hours after injury. Thereafter, the total concentration decreased to as low as 1.0 mgm. per 100 cc. on the 7th day. The cholic acid fraction decreased from the original level of 80 per cent to 40 on the second day, 67 on the fourth,

50 on the seventh and to 43 on the tenth day after injury. Only once on the eighth day did the original bile acid ratio return.

The maximal increases in total urinary concentration of bile acids paralleled those found in biliary obstruction. Increased concentrations were voided even when the blood total and cholic acid fractions were decreased, and in the absence of bilirubinemia and bilirubinuria. The maximal shifts toward "non-cholic" acid were not as great as those found in biliary obstruction (maxima, 70 per cent versus 55 per cent), although the total bile acid concentrations were practically equal.

COMMENT. The "non-cholic" acid fraction, essentially desoxycholic acid was found in the blood of normal dogs in amounts of 10 to 45 per cent of the total bile acids and in the urine it was virtually absent (0-10 per cent).

The total bile acid and the cholic acid concentrations were increased in the blood and urine after biliary obstruction. The increase in the blood was at times entirely due to cholic acid (100 per cent). The cholic acid content in the blood was always greater than before biliary obstruction. In the urine, on the other hand, the "non-cholic" acid per cent rose after obstruction. The explanation for this unexpected finding in the urine is not fully apparent. Ordinarily, cholic acid has been found to be more freely excreted in the urine than desoxycholic acid (10). It is suggested that after complete occlusion of the essential excretory channel for desoxycholic acid in the dog, the biliary passages,[4] the kidney takes over this function in a larger measure and "non-cholic" acid becomes more freely excreted in the urine than normally. Thus, relative increases of cholic acid in the blood and "non-cholic" acid in the urine ensues. This explanation is more plausible than the assumption of a selective interference in the excretion of cholic acid by the kidney after biliary obstruction. The excretion of a markedly increased concentration of total bile acids and cholic acid in the urine indicates that renal damage is not responsible for the high level of cholic acid in the blood.

In contrast, acute liver damage induced by a single dose of carbon tetrachloride produced a marked decrease in total concentration of bile acid in the blood after an initial transitory rise for 24 hours. The cholic acid ratio showed a tendency, generally, to decrease from its original level. This change occurred even without bilirubin retention in the blood but with microscopic evidence of liver damage. On several days the bile acid concentration in the urine was markedly elevated, but the "non-cholic" acid ratio did not reach that found after complete biliary obstruction.

An increased concentration of total bile acids may occur in the urine simultaneously with a decreased concentration in the blood. Bollman and Mann (5) gave carbon tetrachloride to a dog with obstructive jaundice.

[4] In dog's urine, the bile acid content is virtually all in the form of cholic acid.

The blood level of cholic acid estimated by the Gregory-Pascoe method dropped from 30 mgm. to 5 mgm. per 100 cc. but the urinary total in 24 hours only dropped from 1060 to 510 mgm. The urinary level remained markedly elevated although the blood concentration was markedly reduced.

The "hemolytic" values of bile acids in the blood varied considerably in different normal dogs and on various days. Nevertheless, the rises in these values after liver injury, though slight, are probably significant for the following reasons: The "hemolytic" values in the blood, and those in the urine to lesser degree, in each experiment rose above the normal at the same time period after the liver injury. Simultaneously with the increases in "hemolytic" values, a qualitative change from a preponderance of cholic to "non-cholic" acid was observed. It is this shift to "non-cholic" acid, 7.6 times more hemolytic than cholic acid, after liver damage by carbon tetrachloride, which may account for the slight *increase* in "desoxycholate

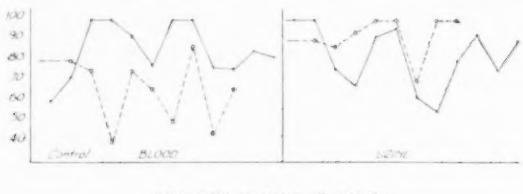



Fig. 3. Cholic acid partition (per cent) (dogs 8 and 14). x------x-----x Biliary obstruction. o------o------o Liver damage.

hemolytic equivalents" while simultaneously the absolute total bile acid and cholic acid concentrations in the blood show a *decrease*.

The formation of bile acids is an intrinsic function of the liver. Altered quantitative relationships between types of bile acids in body fluids may be an expression of altered liver function and may occur even before jaundice develops. Available data (1, 2, 5) indicate that liver damage may reduce bile acid formation. The bile acid content of bile (1, 2) and the amount of cholic acid in the blood and bile (5) are reduced after carbon tetrachloride poisoning. Facts pertaining to desoxycholic acid under these circumstances are not available. The formation of this bile acid may also be depressed. It is probable that the increase in "non-cholic" acid observed by us was not due to increased formation of this type of bile acid under pathological conditions but rather to a relative shift in production of cholic or desoxycholic acid. The excretion of an increased concentration of bile acids in the urine observed by us after liver injury by carbon tetrachloride is not incompatible with the previous findings of others, of reduced bile acid formation by the liver after poisoning, since

the total amounts of bile acids excreted in the urine are insignificant compared with the amounts eliminated in the bile.

SUMMARY

The cholic acid fraction constitutes 55 to 90 per cent of the total bile acid content of the blood of normal dogs as estimated by a combination of hemolytic and colorimetric methods: the remainder exists probably in the form of desoxycholic acid. In the urine, the bile acids are normally entirely or mainly (90 per cent) cholic acid.

Acute liver injury induced by a single dose of carbon tetrachloride decreased the total bile acid content of the blood and generally the cholic acid fraction. Simultaneously, the total bile acid concentration in the urine was usually increased.

Experimental complete biliary obstruction produced a marked increase in the total bile acid content and the cholic acid fraction in the blood. Simultaneously, the total bile acid and cholic acid concentrations in the urine rose markedly but the relative cholic acid fraction of the total was decreased from the original level.

REFERENCES

(1) Smyth, F. S. and G. H. Whipple. J. Biol. Chem. **59:** 623, 1924.
(2) Whipple, G. H. and H. P. Smith. J. Biol. Chem. **89:** 727, 1930.
(3) Greene, C. H., C. H. Fredrickson and W. Walters. J. Clin. Investigation **9:** 295, 1930.
(4) Ravdin, I. S., C. G. Johnston, C. Riegel and S. L. Wright. J. Clin. Investigation **12:** 659, 1933.
(5) Bollman, J. L. and F. C. Mann. This Journal **116:** 214, 1936.
(6) Josephson, B. Biochem. J. **29:** 1519, 1935.
(7) Lichtman, S. S. J. Biol. Chem. **107:** 717, 1934.
(8) Schmidt, L. H. This Journal **120:** 75, 1937.
(9) Gardner, G. H., R. C. Grove, R. K. Gustafson, E. D. Maire, M. J. Thompson, H. S. Wells and P. D. Lamson. Bull. Johns Hopkins Hosp. **36:** 107, 1925.
(10) Lichtman, S. S. This Journal **117:** 665, 1936.

# THE EFFECT OF ACUTE HEMORRHAGE ON ABSORPTION FROM THE SMALL INTESTINE[1]

EDWARD J. VAN LIERE, DAVID W. NORTHUP AND CLARK K. SLEETH

*From the Department of Physiology, West Virginia University, Morgantown, West Virginia*

Received for publication June 17, 1938

It has been shown in previous papers (1936a, 1936b) that anoxic anoxia influences the absorption of distilled water and of sodium chloride solution from the small intestine in the mammal. In order to extend these observations further the effect of a different type of anoxia was studied, namely, anemic anoxia, on the absorption from the small intestine.

METHODS. Approximately the same methods were used as those reported in previous papers (1936a, 1936b). Dogs were used which had been starved 48 hours previous to the experiment; they were given sodium barbital (190 mgm. per kilogram) intravenously. Two animals were paired as near the same weight and age as possible; one animal was used as a control and the other was bled 3.2 per cent of its body weight.

The intestines were exposed by a midline incision. With the exception of the duodenum approximately the entire small intestine was used as a loop. The lengths of the loops were made identical by acutal measurement. The effect of hemorrhage on absorption of distilled water, isotonic chloride solution and isotonic glucose solution was studied. Each substance was studied separately.

Ten pairs of animals were used when the absorption of distilled water was studied; an equal number were used when isotonic sodium chloride solution was studied. Fifteen pairs, however, were used when isotonic glucose was the substance placed in the intestine. Thus 70 animals were used for the entire problem.

A measured amount of the substance to be studied was placed in the intestinal loop; exactly the same amount was used in the control as in the experimental animal. The distilled water was left in the intestine for 30 minutes, the isotonic chloride solution for 40 minutes and the isotonic glucose solution for 90 minutes. These periods were chosen to give approximately 50 per cent absorption in the control group in each case. At the end of the allotted time the contents of the intestine were removed and measured. The actual content of the solute present was determined

[1] Aided by a Grant of the Ella Sachs Plotz Foundation.

by the Van Slyke modification of the Volhard method for the chloride and by the Folin-Wu method for the glucose.

RESULTS. The accompanying table shows that the control animals absorbed more water than did the animals which sustained a hemorrhage. When normal sodium chloride solution was placed in the small intestine, just the opposite results were obtained, that is, more fluid as well as solute was absorbed by the animals which had been bled. The absorption of isotonic glucose solution was not affected by hemorrhage.

DISCUSSION. It is known that fluids from the surrounding tissues pass into the blood stream following a hemorrhage, for the body attempts to bring the plasma volume up to normal. It might be reasonable to suppose that absorption of water from the intestine would be much faster after hemorrhage. It has been shown, however, (Wells, 1932) that the ab-

TABLE 1

*Effect of hemorrhage (3.2 per cent of body weight) on absorption from the small intestine*

| SUBSTANCE | ABSORPTION OF FLUID | | | | ABSORPTION OF SOLUTE | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Hemorrhage | Difference | p† | Control | Hemorrhage | Difference | p† |
| Distilled water | 68.5 | 40.1 | −28.4* | < .01 | | | | |
| 0.9 per cent NaCl | 47.6 | 81.0 | +33.4* | .015 | 58.1 | 81.0 | +22.9* | < .01 |
| 5.5 per cent glucose | 62.9 | 54.1 | − 8.8* | .29 | 68.2 | 61.1 | − 7.1* | .34 |

Ten pairs of dogs were used for each substance except glucose; here 15 pairs were used.

* A minus sign indicates reduction of absorption by hemorrhage.

† Probability of difference occurring by chance. Should be less than 0.05 for significance (Fisher).

sorbing force is proportional to the protein concentration of the blood. It is well known that after hemorrhage there is a diminution of the blood proteins. This could explain why it was that the animals which had sustained a hemorrhage absorbed less water than did the control animals.

Another factor which probably deserves consideration is that distilled water is not a normal agent at the level of the jejunum or ileum. Distilled water may well be toxic to the intestinal epithelium. It doubtless could cause the cell to swell and produce a mechanical block or it might change the permeability, or do both. There is some evidence that the latter actually takes place (Visscher, 1938). It is conceivable that by its irritant action it could cause the epithelial cells to secrete. While all of these factors may play a part in absorption of water when placed in the lower part of the small intestine, they obviously are not so important when the animal is subjected to anoxic anoxemia as has been shown in a previous paper (Van

Liere, David and Lough, 1936). It is quite apparent that the effect of anoxia on absorption produced by hemorrhage has an entirely different mechanism than that produced by simple anoxemia.

The most plausible reason for the fact that the animals which had suffered a hemorrhage absorbed more isotonic salt solution than did the control animals is that during hemorrhage there was a depletion of chlorides throughout the tissues of the body. When isotonic sodium chloride was placed in the intestine there was a higher diffusion gradient and the salt passed into the blood stream more rapidly. The results obtained in studying the absorption of salts were not unexpected, since as previously mentioned the body attempts to restore the blood plasma after a hemorrhage. The anoxia produced by the hemorrhage apparently did not interfere with the absorption of the sodium chloride. When the effect of anoxic anoxemia, however, was studied on the absorption of sodium chloride it was found that even very mild degrees of anoxemia (15.32 per cent oxygen) diminished absorption.

The absorption of glucose was not affected by hemorrhage. Since glucose absorption presumably involves a phosphorylation process (Verzar and McDougal) rather than an oxidative process, the results obtained would not be unexpected. In studying the effects of anoxemia on the absorption of isotonic glucose solution it has been found that absorption is not influenced by the diminution in the amount of oxygen (unpublished work). Thus neither anoxia due to hemorrhage nor anoxia due to simple lack of oxygen in the blood influences the absorption of glucose from the intestine.

In a number of animals a blood pressure tracing from the carotid artery was taken throughout the course of the experiment. The severe hemorrhage, of course, produced a decided drop in blood pressure; the lowest point it reached in an average of 20 dogs was 30 mm. Hg. As soon as the hemorrhage ceased the blood pressure gradually began to rise and at the end of 40 minutes it had returned to within about 38 mm. of the normal.

Since the sodium chloride solution was absorbed more readily in the animals which had sustained a hemorrhage than was distilled water it was felt that the sodium chloride solution might have raised the blood pressure more rapidly than did the distilled water and this might explain the difference in the rates of absorption. This, however, was found not to be true, for the blood pressure rose approximately at the same rate whether the gut contained sodium chloride solution or distilled water.

The circulatory factor then as a possible mechanism must not be overemphasized to explain the results set forth in this paper; although less water was absorbed from the small intestine after hemorrhage more sodium chloride solution was absorbed and the absorption of isotonic glucose solution was unaffected. The circulatory factors, as nearly as could be

determined, were comparable in each instance, but in spite of this the rate of absorption of each substance studied was influenced differently by the hemorrhage.

## SUMMARY AND CONCLUSIONS

It was found that dogs which had been bled 3.2 per cent of their body weight absorbed less distilled water from the small intestine than did the control animals. Isotonic chloride solution, however, was absorbed more quickly from the intestine in the dogs which had sustained a hemorrhage. Lastly, hemorrhage had no effect on the absorption of isotonic glucose solution from the small intestine.

Some of the possible mechanisms which might explain the results are discussed.

## REFERENCES

VAN LIERE, E. J., N. A. DAVID AND D. H. LOUGH. This Journal **115:** 239, 1936.
VAN LIERE, E. J. AND C. K. SLEETH. This Journal **117:** 309, 1936.
VISSCHER, M. B. Personal communication.
WELLS, H. S. This Journal **101:** 434, 1932.
VERZAR, F. AND E. J. MCDOUGAL. Absorption from the intestine. Longmans, Green & Co., London, 1936.

# THE EFFECT OF DESTRUCTION OF THE SPINAL CORD ON THE ARTIFICIAL PRODUCTION OF HYPERTENSION IN DOGS[1]

FRANK GLENN AND EARL P. LASHER

*From the Department of Surgery of the New York Hospital and Cornell University Medical College*

Received for publication June 11, 1938

In an experimental study of hypertension recently published (1), it was demonstrated that animals with an established hypertension subjected to destruction of the spinal cord below the level of $C_6$, exhibited a primary fall in blood pressure followed within a period of a few days by a rise which within a few weeks had reached a level above normal but not equal to the hypertensive level maintained prior to destruction of the cord. In summarizing the report of this study the authors state that "it would appear from these experiments that destruction of the spinal cord below the level of $C_6$ does not profoundly alter the blood pressure in experimental hypertension produced by partial constriction of the renal arteries."

In the course of an additional series of experiments the purpose of which has been to determine, if possible, the various factors concerned in the maintenance of the blood pressure in essential hypertension, the spinal cords in a small series of dogs have first been destroyed below the level of $C_5$ and then an attempt made to produce hypertension by the method of Goldblatt. It is the purpose of this report to record the effects of destruction of the cord on the development of experimental hypertension in six animals.

METHOD. After the satisfactory production of a Van Leersum carotid loop, careful daily blood pressure observations were made over a period of two weeks or more to determine the average normal blood pressure of the animal. A laminectomy was then performed in the lower cervical region. The spinal cord was sectioned at $C_5$ or $C_6$ and the portion below the section destroyed with a soft solder rod. This procedure was invariably accompanied by a fall of blood pressure to a very low level. The animals were nursed with great care until they recovered from the shock of operation and until the blood pressure approached the normal level. This usually required several days. When the renal arteries were partially occluded, the blood pressure gradually rose to hypertensive levels. The

[1] Supported by a grant from the John and Mary Markle Foundation.

course of this hypertension did not vary materially from the course observed in animals with intact spinal cords. The protocols of the six experiments are as follows:

*Experiment I.* The normal blood pressure determined over a period of 65 days was 140 mm. Hg. Laminectomy and destruction of the cord; sharp fall in blood pressure to a level that was imperceptible by the mercury manometer carotid loop method; manifestations of surgical shock were present for three to six hours after operation followed by a gradual rise of blood pressure within four days to the normal level of 140 mm. Hg; constriction of the renal arteries; rise of blood pressure to 280 mm. Hg four days later; a slight fall of blood pressure preceding death eight days later.

*Experiment II.* The normal blood pressure determined over a period of 105 days was 120 mm. Hg. Laminectomy and destruction of the cord; sharp fall of blood pressure to a level that was imperceptible by the mercury manometer carotid loop method; manifestations of surgical shock were present for two hours after operation followed by a gradual rise of blood pressure over a period of five days to 120 mm. Hg; constriction of the renal arteries; rise of blood pressure over a period of four days to a level of 220 mm. Hg; gradual fall in blood pressure to a level of 150 mm. Hg 21 days after constriction of the renal vessels. The secondary constriction of the renal arteries was followed by an elevation of blood pressure to 190 mm. Hg within three days accompanied by manifestations of acute hypertension. Death resulted five days after secondary constriction.

*Experiment III.* The normal blood pressure determined over a period of 20 days was 130 mm. Hg. Following laminectomy and cord destruction, the blood pressure fell to 55 mm. Hg and gradually returned to the previous normal level over a period of six days. Constriction of the renal arteries was followed by a rise in blood pressure to a level of 225 mm. Hg within three days. During the following 25 days the blood pressure gradually fell to a level of 150 mm. Hg. The degree of constriction was again increased. The blood pressure rose to almost 200 mm. Hg within four days. The hypertensive level was maintained for 33 days when the animal was subjected to a left nephrectomy following which it died three days post-operatively.

*Experiment IV.* The normal blood pressure determined over a period of 32 days was 130 mm. Hg. Laminectomy and cord destruction were accompanied by a fall of blood pressure to approximately 8 mm. Hg as measured by the mercury manometer carotid loop method; gradual rise of blood pressure to normal level over a period of seven days; partial constriction of the renal arteries was followed by an elevation of the blood pressure to 200 mm. Hg over a period of 24 hours. Because the animal had developed symptoms of uremia the degree of constriction was diminished. The blood pressure then fell over a period of two days to the normal level of 130 mm. Hg. The animal was then sacrificed.

*Experiment V.* The normal blood pressure determined over a period of 15 days was 130 mm. Hg. Laminectomy and cord destruction were followed by a fall of blood pressure to a shock level from which the animal recovered over a period of five days. The renal arteries were then constricted, followed by a rise of blood pressure to 190 mm. Hg within two days. The blood pressure then fell again but gradually rose over a period of 20 days to a level reaching 220 mm. Hg. The animal was then sacrificed.

*Experiment VI.* The normal blood pressure determined over a period of 54 days was 130 mm. Hg. The animal was subjected to a laminectomy and cord destruction which were followed by a fall of blood pressure to a level that was imperceptible by the mercury manometer carotid loop method. Manifestations of surgical shock

were present for four hours after operation followed by a gradual rise of blood pressure within two days to 115 mm. Hg. Constriction of the renal arteries with an elevation of the blood pressure to 180 mm. Hg within four days. The blood pressure then fell over a period of a few days to 150 mm. Hg and the renal arteries were further constricted; elevation of blood pressure to 220 mm. Hg within two days. One day after reaching this level, the animal died in acute hypertension.

SUMMARY OF CLINICAL AND PATHOLOGICAL FINDINGS: To avoid repetition these findings in the various experimental animals are summarized.

The clinical findings and course were such as to leave no doubt that there had been complete destruction of the cord below the ligature ($C_5$).

*Spinal cord.* The appearance of the spinal cord at autopsy in the animals subjected to destruction in the manner described, varied with the time which had elapsed after the procedure. Within a period of 10 days after the operation the vertebral canal contained extensive blood clots and the cord was softened and extensively infiltrated with hemorrhage throughout. After four weeks the hemorrhage in the vertebral canal had diminished but had not entirely disappeared. The spinal cord showed definite destruction. Sections of the cord were taken at various levels ($C_1$, $C_6$, $D_1$, $L_1$) and fixed in Mueller's fluid and stained with osmic acid; other sections were stained with hematoxylin and eosin. The sections show marked edema, destruction and disappearance of the fiber tracts. For the most part the sections showed complete anatomical destruction of the cord and so extensive were the changes that there can be little doubt of its complete loss of function, as was manifest by the clinical observations of these animals.

*Heart.* The cardiac muscles of three of the six animals suggested mild cardiac hypertrophy; in three no gross changes were observed.

*Kidneys.* The kidneys showed the anatomical changes noted in other dogs exhibiting hypertension secondary to constriction of the renal arteries. These have been described by Child (2) and Goldblatt (3) and can probably best be interpreted as focal infarction with varying degrees of degeneration and repair.

*Liver, pancreas and spleen.* There were no significant changes in these organs other than those found in the arterioles and small arteries. The vascular lesions were varied, being of an acute inflammatory nature in those animals in which the constriction of the main renal arteries had been too great, and consisting chiefly in thickening of the media in the animals which had maintained a hypertension for three weeks or more.

DISCUSSION. The experiments herein described were undertaken with the object of determining, if possible, the rôle of the central nervous system in the production of experimental hypertension. On the basis of experimental and clinical evidence it would appear that destruction of the spinal cord below the fifth cervical vertebra should eliminate the part

of the central nervous system which is chiefly concerned with the maintenance of the level of blood pressure, and presumably with hypertension. These experiments show that in dogs, the destruction of the spinal cord below the level of the fifth cervical vertebra does not materially affect the production of experimental hypertension.

An interesting fact concerning the blood pressure is apparent in these animals. This fact is most pronounced in experiment III in which extreme fluctuations occurred. Since the same routine was used each day in the care of all dogs, we attribute the lability of the blood pressure to the loss of spinal vasomotor reflexes as observed by Heymans et al. (4). Herman, Morin and Vial (5) have noted a similar lability in the normal dog in which the dorso-lumbar cord had been removed by a curette. They suggest that the loss of reflex spinal regulation may render the vascular bed more susceptible to the influence of humoral factors.

## REFERENCES

(1) Glenn, F., C. G. Child and I. H. Page. This Journal **122:** 506, 1938.
(2) Child, C. G. J. Exper. Med. **67:** 521, 1938.
(3) Goldblatt, H. J. Exper. Med. **67:** 809, 1938.
(4) Heymans, C., J. J. Bouckaert, S. Farber and F. Y. Hsu. This Journal **117:** 619, 1936.
(5) Hermann, H., G. Morin and J. Vial. Arch. internat. de physiol. **44:** 125, 1937.

# ORGAN HYPERTROPHY FOLLOWING INJECTIONS OF THYREOTROPIC HORMONE

ROSEMARY MURPHY, STEPHANIE LOWTHER AND LUCIA PAGNIELLO

*From the Department of Zoology and Physiology, Wellesley College*

Received for publication June 4, 1938

The present paper is a report of the increase in size of the thyroid, heart, and kidneys of albino rats that have received injections of the thyreotropic hormone of the anterior lobe of the pituitary gland. Since hypertrophy of the heart and kidneys occurs when thyroid gland preparations are administered to rats (Hoskins, 1; Cameron and Sedziak, 2; MacKay and MacKay, 3), it would seem logical that stimulation of the thyroid gland by extracts of the whole pituitary or by thyreotropic preparations should, likewise, result in an enlargement of the heart and kidneys. However, Hoskins (1) reports that dried pituitary glands do not produce a significant variation in the size of these organs. In view of the fact that rats are not very sensitive to pituitary thyreotropic hormone and that a regression of the response occurs after continuous administration (Anderson and Collip (4) and Smith (5)), it seemed possible that failure to obtain heart and kidney hypertrophy could be ascribed to an insufficient dosage or to an experimental period that extended into the refractory stage.

Consequently, the following experiments were conducted to determine the effect of rather large doses of a preparation of the thyreotropic hormone administered for a short period of time.

EXPERIMENTAL PROCEDURE. Male and female albino rats (3–4 weeks old) from an inbred strain reared in this laboratory were used for the experiments. They were kept in wire cages and food consumption and body weights were determined daily. The diet for all of the animals was similar to that used by MacKay, Smith, and Closs (6) in their study of desiccated thyroid and was made up as follows: commercial casein, 20 per cent; cornstarch, 36 per cent; dried brewer's yeast (Vita-Food Red Label), 10 per cent; lard, 20 per cent; cod liver oil (Patch), 10 per cent; and salt mixture, 4 per cent. This diet was supplied *ad libitum* to all the animals with the exception of the restricted control groups in which case the food was limited in quantity so that the rate of growth would approximately equal that of the experimental animals.

For each individual experiment litter-mates were kept as controls for the experimental animals which were given daily for 10 days subcutaneous

TABLE 1

*Comparison of organ weights of injected animals with restricted and unrestricted controls*

| SERIES | GROUP | DOSE | NUMBER OF ANIMALS | | FINAL WEIGHT | SURFACE AREA | THYROID | | HEART | | KIDNEY | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Actual | per 100 sq. cm. | Actual | per 100 sq. cm. | Actual | per 100 sq. cm. |
| | | *cc.*† | *M* | *F* | *grams* | *sq.cm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| I | C | | 10 | | 93 | 225 | 6.6 | 2.93 | 428.5 | 190.4 | 586.7 | 260.7 |
| | E-1 | .02 | 10 | | 97 | 232 | 7.0 | 3.02 | 448.6 | 193.3 | 573.5 | 247.2 |
| | E-2 | .04 | 10 | | 94 | 227 | 9.4 | 4.14 | 435.2 | 191.7 | 585.7 | 258.0 |
| II | C | | 5 | | 77 | 199 | 5.2 | 2.61 | 408.8 | 205.4 | 506.8 | 254.7 |
| | E | .3 | 5 | | 70 | 186 | 10.2 | 5.48 | 424.5 | 228.2 | 550.1 | 295.7 |
| III | C | | 5 | | 74 | 194 | 5.44 | 2.80 | 382.8 | 197.3 | 402.9 | 207.7 |
| | E | .3 | 5 | | 69 | 185 | 9.96 | 5.38 | 401.6 | 217.0 | 454.9 | 245.9 |
| IV | C | | 1 | 3 | 85 | 211 | 6.3 | 2.98 | 451.6 | 214.0 | 505.2 | 239.4 |
| | E | .6 | 1 | 4 | 61 | 170 | 11.6 | 6.82 | 382.2 | 224.8 | 525.5 | 309.1 |
| V | C | | 4 | 1 | 81 | 205 | 6.1 | 2.97 | 412.1 | 201.0 | 481.8 | 235.0 |
| | R | | 3 | 1 | 62 | 172 | 6.1 | 3.55 | 290.8 | 169.1 | 380.4 | 221.1 |
| | E | .6 | 4 | 1 | 65 | 177 | 11.4 | 6.44 | 391.0 | 220.9 | 524.8 | 296.5 |
| VI | C | | 2 | 4 | 85 | 211 | 6.9 | 3.27 | 419.3 | 198.7 | 498.9 | 236.4 |
| | R | | 4 | 4 | 62 | 172 | 5.7 | 3.31 | 312.5 | 181.7 | 372.2 | 216.4 |
| | E* | .5 | 4 | 3 | 58 | 164 | 13.3 | 8.11 | 348.8 | 212.7 | 619.6 | 377.8 |

C, unrestricted controls; R, restricted controls; E, injected with Antuitrin-T; E*, Antuitrin-T plus trypan blue.

† 1 cc. of Antuitrin-T stated as containing 50 guinea-pig units.

TABLE 2

*Degree of hypertrophy following injections of antuitrin-T expressed as per cent increase over unrestricted controls*

| SERIES | DOSE | NUMBER OF PREPARATION | THYROID | HEART | KIDNEY | MONTH OF EXPERIMENT |
|---|---|---|---|---|---|---|
| | *cc.* | | *per cent* | *per cent* | *per cent* | |
| I | 0.02 | 096485-A | 3.1 | 1.52 | −5.2 | October 1937 |
| | 0.04 | 096485-A | 41.3 | 0.68 | −1.0 | October 1937 |
| II | 0.3 | 096485-A | 109.9 | 11.1 | 16.1 | December 1937 |
| III | 0.3 | 096485-A | 92.1 | 9.98 | 18.4 | January 1938 |
| IV | 0.6 | 096635-A | 128.8 | 5.04 | 29.1 | April 1938 |
| V | 0.6 | 096635-A | 116.8 | 9.9 | 26.2 | May 1938 |
| VI | 0.5* | 096485-A | 148.0 | 7.04 | 59.8 | May 1938 |

* Plus injections of trypan blue.

injections (intraperitoneal injections in series III) of varying quantities of a preparation of the thyreotropic hormone (Antuitrin-T[1]). The experimental animals in series VI received in addition an intraperitoneal injection of 1 cc. of a 0.5 per cent solution of trypan blue in mammalian Ringer's solution on the day preceding the first injection of Antuitrin-T and 0.5 cc. of trypan blue each alternate day thereafter.

On the day following the last injection the animals were killed with gas and the thyroids, heart, and kidneys removed and weighed. For purposes of comparison the weights of the organs were expressed on the basis of surface area calculated from the formula, $S$ equals $11.36 \times W^{2/3}$ (7) where $W$ is the final weight minus 5 per cent (correction for the weight of intestinal contents (MacKay and Cockrill (8)).

The doses, preparations used, time of year during which the experiments were conducted, actual weights of the organs, and relative degrees of hypertrophy are given in table 1 and table 2.

Results and discussion. It can be seen that 0.02 cc. of Antuitrin-T apparently was a sub-minimal dose for the rat since there was no appreciable change in the thyroid weight; 0.02 cc. of this preparation has been reported to produce a measurable effect in the guinea pig (Starr and Rawson, 9). The 0.04 cc. dose produced a slight increase in the size of the thyroid with no accompanying variation in the size of the other organs. With 0.3 cc., a fairly large dose on the basis of the guinea-pig standard, a significant increase in the size of the thyroids occurred accompanied by a smaller enlargement of the heart and kidneys. It should be noted that a greater hypertrophy occurred in the kidneys than in the heart. With 0.6 cc. of Antuitrin-T a still greater hypertrophy resulted in the thyroid and kidneys, although the heart weight showed no further increase. That the enlargement of the kidney is not merely an apparent increase caused by a retardation of body weight unaccompanied by a comparable retardation of kidney weight is shown by a comparison of the experimental animals in series V and VI with their restricted controls.

The thyroids and kidneys of the experimental animals in series VI injected with 0.5 cc. of Antuitrin-T and "dye-blocked" with trypan blue showed the largest increase in size of any of the groups. The extract used in this series was several months older than that used in the other series and, therefore, might be expected to have lost some of its potency. However, the increase in size of the kidney was over 3 times that obtained with 0.3 cc. and more than twice that obtained with 0.6 cc.

Loss of sensitivity to pituitary extracts has been ascribed to the development of an antagonistic substance, possibly of an antibody nature (Gordon, 10). This concept led Gordon *et al.* (10, 11) to attempt to prevent

[1] We wish to express our gratitude to Dr. Oliver Kamm of the Parke, Davis & Co. who so generously supplied us with the Antuitrin-T used in this investigation.

the production of the inhibitory substance by splenectomy and by "dye-blocking" the reticulo-endothelial tissue. They report success with gonadotropic and thyrotropic hormones with this procedure. However, they state that the inhibitory substance has not been detected in the blood before the 10th to the 20th day which theoretically should eliminate inhibitory substances as a factor in our results since our experiments were terminated after the 10th injection. Nevertheless, the enhanced potency of the extract when injected into the "blocked" animals of series VI indicates that the inhibitory substance is present in the rat before the 10th day of injection and that failure to detect it probably results from a lack of sensitivity of the methods used.

The failure of the heart to respond in a manner similar to the kidney is of considerable interest. As previously noted enlargement of the heart as well as the kidney occurs following thyroid administration. It might be expected that the same effect would occur with pituitary extracts. However, even in the "blocked" rats the hypertrophy of the heart did not parallel that of the kidney. At present no satisfactory explanation can be offered for this result.

## CONCLUSIONS

Experiments have been reported which indicate that in spite of the refractoriness of the rat to thyreotropic hormone a slight increase in the size of the heart and a greater increase in the size of the kidney occur when sufficiently large doses of a fairly pure thyreotropic extract are administered. The response of the thyroid and kidney can be considerably enhanced by "dye-blocking" the reticulo-endothelial tissue previous to and during the period of injection. The results suggest that an inhibitory substance is present in the rat previous to the 10th day of administration of thyreotropic preparations.

## REFERENCES

(1) Hoskins, E. R. J. Exper. Zool. **21:** 295, 1916.
(2) Cameron, A. T. and F. A. Sedziak. This Journal **58:** 7, 1921.
(3) MacKay, E. M. and L. L. MacKay. J. Nutrition **4:** 33, 1931.
(4) Anderson, E. M. and J. B. Collip. J. Physiol. **82:** 11, 1934.
(5) Smith, P. E. J. A. M. A. **104:** 548, 1935.
(6) MacKay, E. M., F. M. Smith and K. Closs. J. Nutrition **6:** 151, 1933.
(7) Mitchell, H. H. and G. C. Carman. This Journal **76:** 385, 1926.
(8) MacKay, E. M. and J. R. Cockrill. This Journal **4:** 25, 1931.
(9) Starr, P. and R. W. Rawson. Proc. Soc. Exper. Biol. and Med. **35:** 603, 1936-37.
(10) Gordon, A. S. Symposia on quantitative biology **5:** 419, 1937.
(11) Gordon, A. S., W. Kleinberg and H. A. Charipper. Proc. Soc. Exper. Biol. and Med. **36:** 484, 1937.

# THE RELATION OF ESTROGENIC SUBSTANCES TO THYROID FUNCTION AND RESPIRATORY METABOLISM

T. C. SHERWOOD

*From the Department of Anatomy and Physiology, University of Kentucky, Lexington*

Received for publication April 29, 1938

In a series of investigations the author has been able to show that estrogenic substances affect the respiratory metabolism, (1933), (1936), and (1937). Otherwise normal ovariectomized animals given theelin (Parke Davis) and amniotin (Squibb) showed a decrease in respiratory metabolism from 10 to 58 per cent. Ovariectomized animals that were fed desiccated thyroid returned to the normal metabolic level in approximately ten days. Amniotin injections which immediately followed thyroid feeding brought about a return to normal in approximately four days. No measurable decrease in respiratory metabolism was noticed when amniotin was given to thyroidectomized animals after the hypothyroid level had been established.

In view of these findings it seemed desirable to determine the effect of amniotin[1] upon the respiratory metabolism of thyroidectomized animals that had been fed desiccated thyroid.

EXPERIMENTAL PROCEDURE. Nineteen adult ovariectomized rats were thyroidectomized after the normal respiratory metabolism had been established. The metabolic rate was determined at 28°C. and the data calculated in calories per square meter of body surface (Lee, 1929).

When the hypothyroid level had been established, eight animals were administered desiccated thyroid for a period of three days. Each animal was given 1000 milligrams of desiccated thyroid per kilogram of body weight daily. Metabolism determinations were made daily until the hypothyroid level had again been established.

Four of the above animals were then fed desiccated thyroid a second time. These animals were then given three daily doses of 1000 international units of amniotin immediately following the cessation of thyroid feeding.

Eleven animals were administered desiccated thyroid which was immediately followed with amniotin therapy. Each animal received three daily doses of 1000 milligrams of desiccated thyroid per kilogram of body

[1] The amniotin was generously supplied by E. R. Squibb and Sons.

weight. On the fourth day 1000 international units of amniotin were given and continued until three daily doses had been injected.

Results. The normal basal heat production was found to be 33.0 calories per square meter per hour and the decrease in basal heat production following thyroidectomy approximately 30 per cent.

The eight animals used as controls on the thyroid substance showed an increase of 79 per cent in heat production and a return to the hypothyroid level in 24 days. The experimental animals showed an increase of 75 per cent following thyroid feeding and a return to the hypothyroid level in 12 days when amniotin injections immediately followed the thyroid feeding period.

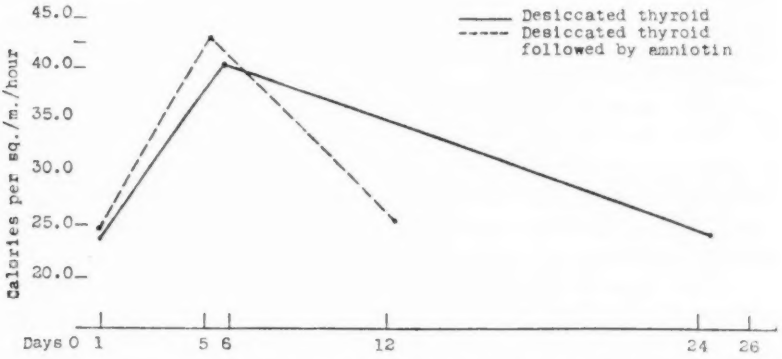



Chart 1. These curves represent the average metabolism for the group of animals that were fed only desiccated thyroid and the group fed desiccated thyroid followed by amniotin injections. All animals were thyroidectomized and ovariectomized.

Chart 1 presents curves on the average metabolism for the group of animals fed desiccated thyroid and the group fed thyroid followed by amniotin injections.

Discussion. Earlier investigators were unable to demonstrate that estrogenic hormones affected respiratory metabolism. This was probably due to the fact that the existing relationship involved several endocrine glands.

Collett, Smith and Wertenberger (1934) found that estrin, in the form of theelin, theelol, or amniotin, affected the basal metabolism in ovariectomized women. Heavy doses produced a fall during administration of the hormone which was succeeded by a rise in the metabolic rate. The data of the present investigation give no indication of results similar to those mentioned above. One might therefore suspect a species difference.

In the present investigation the thyrotropic hormone may have been inhibited by means of amniotin in the non-thyroidectomized animal which

may in turn have caused a decrease in the basal heat production. This did not seem to be the case in the experimental animals where an excess of thyroid substance not produced by the gland must have been neutralized or excreted. The thyrotropic substance apparently was not involved where the amniotin decreased the metabolic rate in thyroidectomized animals fed thyroid. This was apparently a direct hormone antagonism or a direct tissue effect.

The hypophysis may have been inhibited by means of amniotin therapy since the decrease in basal heat production was of the same degree as reported by Foster and Smith (1926) following hypophysectomy.

The reaction was much more rapid than that taking place upon thyroid gland removal. Here the basal heat production did not reach the hypothyroid level until 20 days had elapsed. Approximately 24 days were necessary for the organism to rid itself of the excessive thyroid hormone following thyroid feeding.

Collip's antihormone theory (1934) may account for the findings in the present investigation, provided the time involved is of sufficient length to allow such a reaction to take place. The amniotin had an immediate effect upon the basal heat production. This would indicate that the excess thyroid hormone was rapidly removed and could no longer affect tissue metabolism.

Further investigation is necessary to determine the rôle of the hypophysis in the results obtained. Considerable data have been obtained which indicate that other factors than the hypophysis are involved.

## SUMMARY

Thyroidectomized animals were fed desiccated thyroid for a period of three days. The basal heat production returned to the hypothyroid level in approximately 24 days.

A group of thyroidectomized animals were given desiccated thyroid substance for a period of three days and injected with amniotin for three days following thyroid feeding. The basal heat production returned to the hypothyroid level in approximately 12 days.

The results appear to have been due to a neutralization of the excess thyroid substance or an elimination of this product by means of an antihormone reaction.

## REFERENCES

Collett, M. E., J. T. Smith and G. E. Wertenberger. Am. J. Obstet. and Gynec. **34:** 639, 1937.

Collip, J. B. Ann. Int. Med. **8:** 10, 1934.

Foster, G. L. and P. E. Smith. J. A. M. A. **87:** 2151, 1926.

Lee, M. O. This Journal **89:** 24, 1929.

Sherwood, T. C. and L. M. Bowers. This Journal **115:** 645, 1936.

Sherwood, T. C., M. Savage and J. F. Hall. This Journal **105:** 241, 1933.

Sherwood, T. C., T. M. Wilson and H. Boneta. This Journal **120:** 671, 1937.

# MAMMALIAN PLANTAR REFLEXES IN TERMS OF AFFERENT FIBERS

A. SIDNEY HARRIS

*From the Department of Physiology and Pharmacology, University of Alabama, and Department of Otolaryngology, Washington University, Saint Louis*

Received for publication June 7, 1938

In an earlier study (Harris, 1935) it was shown that in the bullfrog cutaneous afferent fibers of specific functional kinds tend to be grouped in terms of certain properties by which they can be identified, such as conduction rate, threshold to induction shock stimulation, fiber diameter, and locus of skin area supplied. Large, rapidly conducting, low threshold afferent fibers which cause reflex ipsilateral extension are distributed to the following areas: 1, that covering the triceps femoris muscle; 2, dorsal to the gastrocnemius, and 3, the plantar surface. These areas also contain fibers with other functions, but other areas of the limb are not supplied by fibers which perform this function. The prominent reflex evoked by the nerves supplying the skin of the toes, dorsum of the foot and front of the leg is flexion. The afferent fibers which elicit this reflex are smaller, conduct less rapidly, and have a higher threshold to induction shocks than the largest of the cutaneous extensor afferents. They occupy a lower range in the fiber spectrum.

Near threshold faradization of the skin of the planta of the reflex dog in the region of the plantar cushion, especially just proximal to the cushion, evokes a reflex response of plantar flexion, accompanied by extension of the ankle and sometimes of the knee as well. Stronger faradization will evoke this response followed immediately by (or mixed with) withdrawal of the foot by a generalized flexor reflex. Pressure or light pinching of the plantar cushion likewise evokes the plantar flexion and ankle extension reflex while a severe pinch evokes withdrawal which is also accompanied by plantar flexion and appears to have a weak gastrocnemius contraction mingled in with the early part of the reaction.

Experiments with the plantar nerves offer an explanation for the reactions observed in the skin experiments.

METHODS. *Dogs for the experiments were prepared* by 1, decerebration (brain stem section); 2, spinal transection; 3, nembutal (no neural axis operation). The decerebrations were performed aseptically under nembutal one or two days before the experiments. The planes of transection entered dorsally between the superior

and inferior colliculi and emerged ventrally at levels ranging from the mammillary bodies back through the anterior fourth of the pons. Some of these animals showed a high degree of extensor tonus while some were almost flaccid, especially those with the more posterior lesions. Spinal transections were made at about the last thoracic segment. Periods up to fifteen days were allowed to elapse between transection and experiment. In all of these variously prepared animals the reaction to stimulation of the plantar nerves followed a common pattern with only minor differences which will be pointed out.

*The medial plantar* nerve supplies the greater part of the cutaneous innervation of the dog's planta, but has only a small component of fibers to muscle. These are distributed to the first lumbrical and the short flexors (Bradley, 1912). In the distal half of the foot the medial plantar nerve is made up entirely of cutaneous nerve fibers, and the plantar cushion together with the adjacent skin is richly supplied by fibers

Fig. 1. Brain stem of a dog showing the limits of the planes of transection

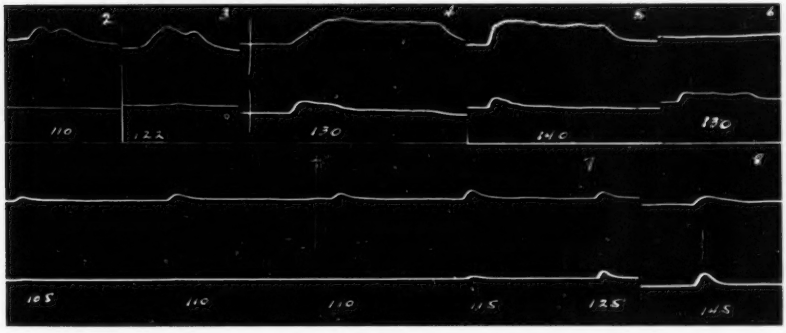
from it. The *lateral plantar* is the main trunk supplying innervation to the muscles of the foot. Just distal to the ankle it passes deep to the short flexor tendons and courses forward in this deep position until it breaks up into many small branches, almost all of which terminate in muscles. Just as it is entering its deep position it gives off a superficial branch which is distributed to the skin along the lateral margin of the foot. From the main trunk there are three small branches which continue forward along the interossei to become superficial at the digits. These supply a few fibers to the plantar cushion and adjacent zone, and join with twigs from the medial plantar to be distributed to the skin of the digits.

Results. *Stimulation of the medial plantar nerve* in all animals with thyratron shocks at 60 per second with near threshold voltage evoked an extensor complex consisting of plantar flexion, ankle and knee extension. The plantar flexion is the most vigorous and most constant component of the response. It is a very durable and persistent reaction, and may be evoked as long as any limb responses are obtainable. Ankle extension is next in vigor and durability: however, it does not occur in all cases, and may be weak when the plantar flexion is quite strong. Only occasionally

is knee extension, or contraction in the quadriceps muscles discernible, and when it occurs extension of the ankle and flexion of the digits are more prominent.

There is slight gradation in the strength of response as the stimulus voltage is increased. The upper myograms in figures 2, 3 and 4 are records made from the achilles tendon showing the changes that occur as stimulus voltage is increased from 110 to 130 per cent of the extensor reflex threshold. The main difference is a greater steadiness of contraction.

The lower myograms in these same records show the simultaneous activity in tibialis anterior, the ankle antagonist to gastrocnemius. The threshold contraction may be seen at 122 per cent of the extensor reflex




Figs. 2, 3, and 4. Extensor and flexor reflexes from the medial plantar nerve. Upper myogram registered from the tendon of Achilles; lower myogram from tibialis anterior. Stimuli, thyratron shocks at 60 per second. Voltages are expressed in percentage of the extensor reflex threshold.

Figs. 5 and 6. Another experiment. In 5 stimuli (60 per second) were applied to the medial plantar, in 6 to the lateral plantar.

Figs. 7 and 8. Stimuli applied to the medial plantar nerve at 1 per second while voltage is increased as indicated.

threshold voltage. The contraction in tibialis never became very strong and was quickly suppressed, perhaps due to inhibition from the extensor reflex arc. Figures 5 and 6, records from another experiment, show this inhibition of the flexor perhaps even better; 5 is a record of the activities of the antagonistic muscles when the medial plantar nerve was stimulated and 6 when a similar degree of contraction in the flexor was evoked via the lateral plantar. This record does not show any activity in the extensor muscle, nor is there an inhibition or simulation of fatigue comparable to the weakening of the flexor activity in 4 or 5. *The superficial branch of the lateral plantar nerve* gives rise to reflex responses similar in every way to those of the medial plantar. However, the reflexes from the *main*

*trunk of the lateral plantar* distal to the point of departure of the superficial branch are very different. The flexor reflex of limb withdrawal constantly occurs. Usually the digits spread and are extended. In no case has an ipsilateral extension reflex from its stimulation been observed. So consistently absent was this reflex in experiments with application of carefully graded thyratron shocks to the deep main trunk of the lateral plantar nerve that a special type of experiment was devised to test whether or not it contained extension reflex afferent fibers. The medial plantar nerve was sectioned at the ankle, and time allowed for recovery of the wound. Then the reflexes were tested by pressure and faradic shocks applied to the plantar cushion. By both of these methods the extension reflex could still be evoked. Again the lateral plantar nerve was exposed and stimulated without yielding an extensor response, but when the small nn. digitorum communes plantares (Ellenberger and Baum) extending forward from the lateral plantar were stimulated the extension reflex appeared again, showing that the deep division of the lateral plantar nerve does contain some fibers of the extensor reflex kind, but that when the nerve is stimulated by induction shocks their activity is masked or inhibited in some way by other fibers in the large mass composing the nerve.

The fibers mediating the two reflex complexes were distinguished by study of their reflex threshold voltages and their conduction rates as shown by the cathode ray oscillograph. The most irritable of the fibers which evoke ipsilateral extension have lower thresholds than do the most irritable fibers which evoke the flexion reflex. This is true whether the fibers being compared are in the same or different nerves. The flexion reflex fibers require a shock voltage about 120 to 130 per cent of the extensor fiber threshold voltage. The data in table 1 may be considered as typical of results in all experiments using rapidly repeated thyratron shocks for stimulation.

When single shocks, or shocks at low frequency are used for stimuli there is, likewise, a distinct threshold difference between the two reflexes but the margin is somewhat narrower than with higher frequency shocks. Figures 7 and 8 show responses to thyratron shocks at one per second. In this case the medial plantar nerve was stimulated, and the flexor threshold was 115 per cent of the extensor. The variation is from about 110 to 120 per cent. That the margin between the ipsilateral extensor and flexor reflex thresholds is narrower with shocks at 1 per second than at 60 per second is in keeping with findings made in the study of reflexes in the bullfrog, and may be interpreted as a consequence of differing summation time relations in flexor and extensor reflex centers. The reflex thresholds at the higher frequency of stimulation may be taken as more nearly approximating the thresholds of the most irritable fibers evoking the respective reflexes (Harris, 1935). This being true the relative thresholds are about 100 for the extensor reflex to 125 for the flexor.

Cathode ray oscillographic measurements of conduction rates in the plantar nerves are easily correlated with the data from reflex threshold measurements. These records were made from excised nerves in an electrode chamber. The tibial nerve was stimulated and the leads to the amplifier taken from the plantar branch being studied, each in its turn. Conduction distances were usually about 90 to 110 mm. In the record registered from the medial plantar nerve (fig. 9) the large potential complex is seen to be made up of two elevation components, the slower seeming to rise from the crest of the faster one. The same relative voltages are found to exist between the thresholds of the elevations as between those of the reflexes. The threshold of the second elevation is 120 to 130 per cent of the first. On a record such as this it is obviously impossible to say

TABLE 1

*Reflex thresholds in typical experiments*

| PREPARATION | NERVE | REFLEX THRESHOLDS | | FLEXOR / EXTENSOR × 100 |
|---|---|---|---|---|
| | | Extensor | Flexor | |
| | | (*Potentiometer units*) | (*Potentiometer units*) | |
| Spinal (1 week) | M.P. | 320 | 405 | 127 |
| | L.P. | None | 400 | 126* |
| Decerebrate | M.P. | 330 | 400 | 121 |
| | L.P. | None | 410 | 124* |
| | Superf. L.P. | 340 | 390 | 115 |
| Nembutalized | M.P. | 335 | 410† | 122 |
| | | | 430 | |

Thyratron shocks at 60 per second used in all experiments. Readings taken in arbitrary voltage units.

* Using extensor threshold of medial plantar nerve.

† In nembutalized animals the flexor reflex threshold is very variable.

just where the beginning of the second component is, or to measure exactly the conduction rate in the fibers giving rise to this elevation. It is possible, however, to measure the conduction rate of the most rapidly conducting fibers contributing to the first elevation, and to estimate those of the second with a fair degree of accuracy. In making this estimation the position of the beginning of the second elevation was located by drawing a line downward parallel to the rising phase of the first elevation from the visible part of the second. When the conduction rates of the most rapidly conducting fibers in each elevation were thus calculated (22°C.) they were found to be 40 and 27 m.p.s. In other experiments at 37 degrees the rates range from 60 to 70 for the faster elevation and from 40 to 50 for the slower.

Figures 10 and 11 show the elevations registered from the lateral plantar nerve, 10 containing the faster elevation alone and 11 showing both. The

threshold of the most irritable fibers in this nerve is lower than that of the most irritable fibers in the medial plantar, being about 90 per cent as high. The conduction rate is also higher than in the most rapidly conducting fibers of the medial plantar. However, the thresholds of the second elevations of the two nerves coincide very closely as do also their rates of conduction.

Figure 12 is the potential record from a small cutaneous branch of the lateral plantar nerve from which no ipsilateral extensor reflex could be obtained. It contains no elevation corresponding to the fastest one in either of the other two nerves, but its main elevation does correspond in threshold and conduction rate to the second elevations in both the medial and lateral plantar nerves.

Figs. 9 to 12. Tracings from action potential contact records made at 22°C.; 9, medial plantar nerve (in the distal half of the foot) conduction distance 90 mm.; 10 and 11, lateral plantar (distal to superficial branch); 10, fastest elevation alone; 11, both main elevations—conduction distance 85 mm.; 12, small lateral plantar cutaneous branch which did not evoke the extensor reflex. Conduction distance 85 mm.

Discussion. In considering these observations it must be remembered that in its distal part, which we are studying, the medial plantar is entirely a cutaneous nerve, and that a great part of the lateral plantar is distributed to muscle. It was shown by Erlanger (1927) that the most rapidly conducting fibers passing to muscle (motor and sensory) conduct more rapidly than fibers passing to skin. Though this has been shown to be not always true in the bullfrog, no exception has as yet been demonstrated in the mammal. The data from the reflex threshold, elevation threshold, and conduction rate observations point to a simple interpretation. The most rapidly conducting, lowest threshold component of the medial plantar action potential is derived from fibers whose reflex effect is ipsilateral extension. These fibers do not conduct quite as rapidly nor have thresholds quite as low as those which give rise to the fastest component of the lateral plantar potential for which no corresponding reflex has been observed. The second component of the medial plantar record is produced by fibers smilar in irritability and conduction rate to those producing the second elevation of the lateral plantar nerve and the most rapid potential component of some of its cutaneous branches. The lowest threshold fibers which evoke the flexion reflex lie within this group. Table 2 presents data on the reflex function and conduction rate relationship as shown in a typical experiment.

The 68 to 44 meter zone indicates that area of the fiber spectrum of the nerves designated within which the ipsilateral extension afferents (except the small component in the deep lateral plantar) are free from mixture with other afferent fibers which control limb activity. The range occupied by extension afferents undoubtedly extends much below 44 meters, overlapping the flexion fiber range. The 78 to 44 meter zone of lateral plantar fibers indicates the area which contains almost entirely motor and muscle sense fibers. Below 44 meters they would be mixed with flexion reflex afferents and perhaps other functional groups.

The apparent differences in the responses of the differently prepared animals were of a very minor nature and may briefly be summarized. In the spinal animal there seems to be a slight narrowing of the margin between the extensor and flexor thresholds, especially with single shock stimuli which require a larger number of fibers to be excited in order to obtain a response. These findings are explainable in terms of the flexor

TABLE 2

*Summary of functions of fibers in distal parts of the nerves as related to rate of conduction in a typical experiment*

| FUNCTION | RANGE OF RATES | NERVE |
|---|---|---|
| | *meters sec.* | |
| Ipsilateral extension | 68 to 44 | M.P. and Sup. L.P. and very small component in deep L.P. |
| Muscle supply (motor and proprioceptive) | 78 to 44 | Lateral Plantar |
| Flexion reflex | 44 and below | All branches |

bias of the spinal animal. With excitation of fibers only in the threshold zone where there is no functional mixture of fibers excited the responses are as distinct as in decerebrate or nembutalized animals, but when the zone of overlapping of the two functional groups of fibers is reached flexion may manifest itself more quickly. The nembutalized animal exhibits a relatively depressed flexion reflex. This is merely an expression of the greater general durability of the extensor reflex against depressing influences. The flexion reflex can be completely suppressed while the extensor response is still quite active, maintaining its low threshold. Strychnine will restore the flexion reflex but does not reverse the extension response to flexion.

The high degree of constancy of response patterns obtained in animals whose conditions of neural bias differ so greatly indicates that the reflex response obtained is determined by the functional kind of afferent fibers excited. By reason of anatomical distribution, the medial plantar nerve contains a dominant share of the cutaneous fibers which serve in the

extensor postural reactions. Further functional isolation of these fibers by distinctive stimuli provides such a degree of purity of kind of excitation in the spinal cord that the response varies relatively little whether the animal be decerebrate, spinal, or partially anesthetized.

The ipsilateral extension and plantar flexion combination is obviously a postural reaction. The movement is never vigorous and is slow to fatigue. That sensory nerves from the planta form a significant part of the postural mechanism is a well established observation. Schoen (1926) evoked the positive supporting reaction (front limb) by stimuli applied to the skin of the pads alone, and Blake-Pritchard (1926) evoked contraction of extensor muscles of the hind limbs by tactile stimuli in some experiments. In some others there was co-contraction and fixation of joints. Human subjects standing in cold salt solution or with the soles anesthetized by immersion in chloroform an hour or longer exhibit a typical Romberg sign (Heyd, 1862) and at one time there was considerable argument that tabetic ataxia was due in large degree to loss of plantar afferent control (Leyden, 1867).

The extensor thrust of Sherrington (1906) appears to be a totally different type of response, though evoked by stimulation of tactile receptors of the planta. In the extensor thrust the most powerful contractions occur in the hip and knee extensors and are of short duration as in galloping. The postural response evoked by medial plantar nerve stimulation has its focus in the ankle extensors and digital flexors and it is a long enduring activity.

## SUMMARY

Faradic stimulation of low threshold cutaneous fibers in the medial plantar nerve or the superficial branch of the lateral plantar gives rise to an ipsilateral extensor response pattern similar to that which occurs when the pads are lightly pressed or stimulated with non-painful induction shocks. This response occurs independently of the neural bias of the animal. The lateral plantar nerve (distal to the superficial branch) is practically lacking in extensor reflex cutaneous afferent fibers.

Higher voltage shocks applied to the medial plantar nerve superimpose a flexor reflex of withdrawal upon the low threshold extensor pattern. Shocks of sufficient intensity to evoke the flexor reflex will evoke it more vigorously and with greater purity if applied to the lateral plantar.

The voltage threshold of the most irritable fibers which evoke the flexor reflex is about 125 per cent of that of the lowest threshold extensor reflex fibers. The most rapidly conducting extensor cutaneous afferents conduct at about 60 to 70 m.p.s. as compared to 40 to 50 for the most rapidly conducting flexor fibers.

The extensor reflex referred to is a slow postural type of response. The rôle of plantar cutaneous afferent fibers in standing is briefly discussed.

## REFERENCES

BLAKE-PRITCHARD, E. A. Pflüger's Arch. **214:** 148, 1926.

BRADLEY, O. C. A guide to the dissection of the dog. Longmans, Green and Co., 1912.

ELLENBERGER, W. AND H. BAUM. Anatomie des Hundes. Paul Parey, 1891.

ERLANGER, J. This Journal **82:** 644, 1927.

HARRIS, A. S. This Journal **112:** 231, 1935.

HEYD, W. H. S. C. Der Tastsinn der Fussohle als Aequilibrirungsmittel des Korpers beim Stehen, iv, 5-22. Tübingen, H. Laupp, 1862.

LEYDEN, E. Virchow's Arch. **40:** 170, 1867.

SCHOEN, R. Pflüger's Arch. **214:** 48, 1926.

SHERRINGTON, C. S. Integrative action of the nervous system. Scribner's, 1906.

# CONCERNING THE METABOLISM OF FAT AND CARBOHYDRATE

JOSEPH L. DONNELLY[1]

*Fort Thomas, Kentucky*

Received for publication May 16, 1938

Clinical studies have shown that fats are fully oxidized to volatile carbon dioxide and water when carbohydrate is oxidized normally. The metabolic relationship between sugar and fat is also shown by the parallel increase in the blood fat and sugar in diabetes and after the use of adrenalin. This has been studied by Ervin (1), Oliver and Haworth and Arnoldi and Collazo (2) who showed that all conditions which lead to glycosuria lead to lipemia as well.

The ability of an animal to utilize glucose varies with its total alkalinity and any increase in acidity is characterized by an elevation of its blood sugar (1, 3). It will be shown that an increase in the blood fat is accompanied by the same changes and that *any increase in the total acidity of an animal is followed by hyperlipemia and hyperglycemia.*

Thus the injection of acid as well as the acidosis which accompanies starvation, have been found to result in a decreased tolerance for sugar and an increased blood fat (4). An increase in acidity, hyperglycemia and hyperlipemia occur in nephritis, diabetes and in acute diseases of the heart and lungs (5). We find an acid urine and lipemia in phlorhizin diabetes and an increase in the lactic acid, sugar and fat in the blood after the ingestion of thyroxin (6). Likewise there is more fat in the venous blood of a working muscle than in that of a muscle at rest (7).

The researches of Araki and Zillessen have shown that any condition which results in inadequate oxygenation of the tissues of an animal results in an increase in its acidity (8). We find that phosphorus poisoning, asphyxia, anemia, arteriosclerosis and the use of such drugs as morphine and the anesthetics, all of which thus cause an increase in acidity, are characterized by hyperlipemia and hyperglycemia (9). The injection of adrenalin is followed by vasoconstriction, so that the hyperlipemia and hyperglycemia which follow its use may be attributed to the increased acidity which is a consequence of the decreased oxygenation of the tissues. It is of interest to note here that the nervous stimuli which increase the blood sugar also increase the blood fat.

[1] This study developed from experiments which were aided by the Committee on Scientific Research of the American Medical Association.

If there is more than a superficial relationship between the hyperglycemia and hyperlipemia which occur whenever there is an increase in the total acidity of an animal we should be able to show that *there is a decrease in the concentration of both fat and sugar in the blood with any increase in the total alkalinity.* We find that this holds true from the clinical researches of Bloor, Mosenthal, Underhill and Murlin (10) who discovered that the blood fat and sugar of diabetic patients varied with the degree of acidosis and that a decrease in the concentration of fat in the blood and an increased tolerance for sugar, followed relief of an acidosis. These studies were confirmed by the experiments of Van de Veld (11) who proved that the injection of sodium bicarbonate was followed by a decrease in the blood lipoids.

We have seen that an increased consumption of oxygen as well as carbohydrate follows an increase in the total alkalinity of an animal (3). *We shall see now that the ability of an animal to oxidize fat varies with its total acidity.*

Two of the agents concerned in the digestion of fat are markedly affected by acid. Thus the activity of lipase and bile is diminished by acid and there is a decrease in the amount of bile secreted in starvation. The oxidation of fat is modified similarly for there is an increase in the production of acetone bodies whenever an increase in acidity occurs, as in starvation, phlorhizin poisoning, gastrointestinal diseases, fevers, after the use of pituitrin, anesthetics and acidifying diets (12). Conversely the acetonurias of diabetes and following diets rich in fat and protein, are decreased after the administration of sodium bicarbonate (13).

Additional evidence that alkali favors the oxidation of fats was found in a consideration of studies on the oxidation of fatty acids and of oils in vitro (14). In both instances the oxidation was accomplished in an alkaline medium, for Dakin found that hydrogen peroxide oxidized fatty acids very easily when they were in the form of the sodium or ammonium salts and Van der Willigan proved that emulsions of oils were oxidized best by a finely divided stream of air, when the mixtures were buffered by alkali.

The quantitative changes in the fat and sugar of the blood and the differences in the ability of an animal to utilize both fat and sugar are also accompanied by physical changes in the tissue fat. For all of the various conditions which evidence an acid intoxication and a decreased ability to completely oxidize fat are characterized also by fatty degeneration. It has been shown that tissue fats are normally held in a very finely emulsified state by protein and that in fatty degeneration there is an enlargement of the individual globules without a quantitative increase in the total fat of the tissue (15).

The significance of this conception of the alteration in the size of the

fat particle advanced some twenty-five years ago by Hooker and Fischer, has since been recognized by a number of workers. Ervin suggested that glycogen stabilizes the emulsion of fat in normal tissue and that its hydrolysis and loss in diabetes or with any acid intoxication results in a coarsening of the emulsion and an overwhelming of the cell with more fat that it can oxidize (1). On the other hand, Bull maintained that cholesterol increases the size of the fat droplet and thus favors the deposition of fat, while phospholipin increases the transportation and utilization of fat by increasing its dispersion (16). Whatever the ultimate mechanism, it may be stated as a general mode of behavior that *there is an increase in the size of the globules of fat in the tissues and a decrease in the oxidation of fat with any increase in the acidity of an animal.* We hold that the incomplete oxidation of fat in an acid intoxication is due to the loss of reactivity which parallels its decreased dispersion.

There is an enormous increase in the exposed surface of a material with any increase in its fineness. For example, a one cubic centimeter cube develops a surface of sixty square meters after it is divided into particles which have a diameter of 0.1 $\mu$. The energy used to accomplish this dispersion is stored in the particles and the increase in surface is attended by a hundred thousand fold increase in the surface energy of the substance. Other forms of energy are also manifest, perhaps through the conversion of this surface energy, for the phenomena of adsorption and the Brownian movement become active and the particles develop an electric charge and greater internal chemical activity as they are divided into smaller and smaller fragments. A dispersed substance behaves differently than the substance en masse.

While we find no rigid quantitative relationship between the degree of fineness of a fatty material and its susceptibility to oxidation, all writers agree that the ignition temperature of any inflammable material decreases with its increasing fineness (17). A block of wood, for instance, ignites with difficulty and burns slowly, but it ignites readily and burns quickly and completely when it is reduced to sawdust. We find many other examples of this in the susceptibility to explosion of finely divided dusts and oils and in the tendency of friable and powdered coals to absorb oxygen and to ignite spontaneously. The ease with which dispersed materials ignite varies with the substance as well as the degree of dispersion, for W. D. Bancroft has shown that aerosols of sugars explode below red heat while aerosols of oils require a higher temperature. It is significant that sugar is likewise oxidized more easily that fats in the living animal.

We have seen that oils may be oxidized by air when they are dispersed in soaps and buffered by alkali and we find further proof that dispersion favors the oxidation of fat in studies on the production of rancidity. For it has been found that rancidity is produced by a direct oxidation of fat

and that bacteria are not necessary for its development. Recent investigations have proven that fats dispersed in porous materials become rancid faster than those stored in large masses and that the finely dispersed fat of homogenized milk becomes rancid more rapidly than that of untreated milk (18). Proof that these phenomena have a counterpart in living matter is found in the decreased ability of an organism to completely oxidize fat in any condition which results in acidosis and a decreased dispersion of tissue fat.

We have seen that the concentration of sugar and fat in the blood are simultaneously increased with any increase in acidity. The belief is common that the oxidation of sugar is necessary for the normal oxidation of fat, but, as Oliver and Haworth have noted, it is difficult to understand why this increase in the oxidizing agent should be accompanied by an increase in the material to be oxidized. It is of interest in this connection that in a study of the induced oxidation of foods, Palit discovered that the oxidation of fats was retarded by carbohydrate and protein, although the oxidation of all foodstuffs was accelerated by alkali and colloidal iron (19).

From a consideration of all of these investigations we conclude that the forces which alter the oxidation of fats are the same as those which alter the oxidation of sugar, and that *the size of the tissue fat globule and the concentration of fat and carbohydrate in the blood vary directly while the utilization of fat and carbohydrate vary inversely with the total acidity of an animal.* We hold that the instability of glucose in alkali is duplicated in the living organism, and that the increased susceptibility to oxidation evidenced by all finely divided materials, is likewise manifest by an effective oxidation of tissue fats whenever their dispersion is increased by an increase in the alkalinity of an animal. Perhaps future investigations will show us that such apparenty unrelated reactions as the retardation of vital processes in an edema and the acceleration of many biological chemical reactions after the addition of (protective colloid containing) tissue extracts are also governed by the degree of dispersion of the reacting materials.

## REFERENCES

(1) Ervin, D. M. J. Lab. Clin. Med. **5:** 146, 1919.
(2) Oliver, T. H. and A. Haworth. Lancet, p. 144, 1923.
Arnoldi, W. and I. Collazo. Ztschr. ges. exper. Med. **40:** 323, 1924.
(3) Donnelly, J. L. This Journal **123:** 448, 1938.
(4) Schulz, F. N. Pflüger's Arch. **65:** 299, 1896.
Elias, H. Biochem. Ztschr. **48:** 120, 1912.
Mansfield, G. Pflüger's Arch. **129:** 46, 1910.
(5) Bing, H. L. and H. Heckscher. Biochem. Ztschr. **149:** 90, 1924.
(6) Hepler, O. Arch. Int. Med. **55:** 979, 1935.
Eichler, O. and R. Saunders. Klin. Wchnschr. **9:** 1618, 1930.

(7) Lafon, G. Compt. Rend. **156:** 1248, 1913.
(8) Araki, T. Ztschr. Physiol. Chemie **15:** 546, 1891.
Zillessen, H. Ibid., p. 387.
(9) Abderhalden, E. et al. Arch. G. Physiol. **26:** 362, 1927.
Morris, R. S. and T. Boggs. J. Exper. Med. **11:** 553, 1909.
Bauer, I. Wien Klin. Wchnschr. **26:** 830, 1913.
(10) Bloor, W. Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **33:** x, 1918.
Mosenthal, H. and J. Killian. Ibid. **55:** xiiii, 1923.
Underhill, F. J. A. M. A. **68:** 497, 1917.
Murlin, J. R. et al. Proc. Soc. Exper. Biol. Med. **14:** 8, 1916.
(11) Van de Veld, J. Compt. Rend. Soc. Biol. **97:** 1825, 1927.
(12) Morris, R. Clinical laboratory diagnosis. Appleton & Co., N. Y., 54, 1923.
Anselmino, K. J. Klin. Wchnschr. **10:** 2380, 1931.
(13) Mosenthal, F. and J. Killian. Loc. cit.
Maignon, F. and E. Knithakis. Compt. Rend. Soc. Biol. **71:** 639; **99:** 604, 1928.
Underhill, L. Loc. cit.
(14) Van der Willigen, P. Verlkroniek **9:** 67, 1936.
Dakin, H. D. J. Biol. Chem. **4:** 233; **5:** 411.
(15) Fischer, M. H. and M. O. Hooker. Fats and fatty degeneration. John Wiley & Sons, N. Y., 1917, pp. 10, 75 and 85.
(16) Bull, H. B. Biochemistry of the lipids. John Wiley & Sons, N. Y., p. 149, 1937.
(17) Brown, C. R. Dissertation, Cath. Univ., Washington, D. C., 1934.
Alexander, J. Colloid chemical principles and applications. Van Nostrand & Co., N. Y., p. 172.
(18) Rabak, W. Oil and fat industry **8:** 873, 1931.
Doerner, W. and A. Widmer. Lait **11:** 545, 1931.
(19) Palit, C. C. J. Phys. Chem. **36:** 2504, 1932.

# CHANGES IN THE CHEMISTRY AND NATURE OF THE CEREBROSPINAL FLUID DURING FETAL LIFE IN THE PIG

LOUIS B. FLEXNER

*From the Department of Anatomy, Johns Hopkins University*

Received for publication June 8, 1938

The cerebrospinal fluid is thought by some to be an ultrafiltrate or dialysate of the blood plasma; by others, to be a secretion involving energy expenditure by the cells of the chorioid plexus. This disagreement largely arises from the difference in emphasis placed upon the deviations of the concentrations of substances in the cerebrospinal fluid from predictions made by Donnan's Law. The dialysate or ultrafiltration theory is tenable only if these apparent deviations are unreal and due to errors of determination. Doubt as to this last point would materially diminish if a phase of ultrafiltration and a phase of secretion could be demonstrated in the same individual. With the possibility in mind that prior to functional maturity of the fetal chorioid plexus, the cerebrospinal fluid might clearly show the characteristics of an ultrafiltrate of the plasma; and at a later stage, the characteristics of a secretion, the chemical relationships between cerebrospinal fluid and blood plasma have been studied at different stages of fetal development.

MATERIAL AND METHODS. These studies were made upon the pig. Most of the material was collected at a slaughter house under conditions previously described (6). In addition sows were bred at the U. S. Animal Husbandry Experiment Station, Beltsville, Md., and their fetuses delivered by Caesarean section, under nembutal anesthesia reinforced with ether. Samples of cerebrospinal fluid and blood were taken only from fetuses with vigorously beating hearts.

Fetal blood was secured by cardiac puncture. Cerebrospinal fluid was obtained by exposing the occipito-atlantoid ligament and performing an occipito-atlantoid puncture. Cerebrospinal fluid was collected directly into a micropipet which consisted of a graduated 1 mm. tube with a hypodermic needle cemented to one end. Samples were measured with the pipet in the horizontal position, and the meniscus was adjusted under a magnifying lens by absorbing fluid onto filter paper from the needle of the pipet.

Samples of 0.1 or 0.2 cc. of cerebrospinal fluid and blood plasma were used for chloride and urea determinations; for sodium determinations,

samples of 0.2 cc. or 0.5 cc., depending upon the method. With crown-rump (C. R.) lengths under 5.0 cm. about 4 fetuses were necessary for the samples; from 5.0 to 9.0 cm., 1 to 3 fetuses were adequate; and above 9.0 cm., a single fetus furnished sufficient material for analysis. When more than one fetus was used for an analysis, the greatest possible quantities of cerebrospinal fluid and blood were taken from each and then the separate cerebrospinal fluid and blood samples were pooled.

The Wilson and Ball (12) method was used for chloride determinations. The majority of the sodium determinations were made by the volumetric method of Ball and Sadusk (1), proteins being destroyed by ashing; a small number of analyses on 0.5 cc. samples using the Butler Tuthill method (3) were added to check the magnitude of the distribution ratios between cerebrospinal fluid and plasma. These two methods gave the same results. Most of the urea analyses were made with the Looney modification (9) of Karr's urease method (8) on protein-free filtrates with direct Nesslerization. The small error which direct Nesslerization introduces into the determination of absolute quantities of urea is apparently cancelled in the calculation of distribution ratios between cerebrospinal fluid and plasma. A second series of analyses were made following the technique for plasma and cerebrospinal fluid given by Merritt and Fremont-Smith (10). The important point of their technique is to use minimal quantities of sodium tungstate and sulphuric acid in preparation of the protein-free filtrate so that excess tungstic acid does not interfere with the activity of urease. In one group of these experiments protein was precipitated with 1 per cent tungstic acid and 1/6 N $H_2SO_4$ and the pH of the solutions adjusted to between 7 and 8 before addition of the urease. This method with Nesslerization either directly or after distillation gave distribution ratios in the fetal pig material of the same magnitude as those obtained by Karr's method, using protein-free filtrates obtained with 2/3 N $H_2SO_4$ and 10 per cent tungstic acid. In a second group, 1 per cent tungstic acid and 1/15 N $H_2SO_4$ were added to the cerebrospinal fluid; this concentration of acid was insufficient to precipitate the proteins and the apparent urea concentration of the cerebrospinal fluid rose to a value approximately equal to or as much as 12 per cent greater than that of the plasma. Finally, the results of the urease method were compared with those given by Beattie's colorimetric xanthydrol method (2). Distribution ratios determined with xanthydrol agreed with those obtained on the protein-free filtrate so that the values found on cerebrospinal fluid samples to which 1/15 N $H_2SO_4$ and 1 per cent tungstic acid were added were discarded as unreliable. Urea determinations gave values reproducible to within 4 per cent; chloride and sodium determinations, values reproducible to within 1 per cent.

The apparatus and solution described by Hastings et al. (7) were used to determine the freely diffusible sodium of plasma. Because of the small

TABLE 1

*Variation with C. R. lengths of distribution ratios between cerebrospinal fluid and blood plasma of fetal pigs*

* $(\ )_F$ and $(\ )^*_P$ refer respectively to concentrations of indicated substances per unit volume cerebrospinal fluid water and plasma water. The numerals in parenthesis in the columns marked "Range" give the number of analyses made for the indicated substance in the indicated C. R. group.

| C.R. LENGTH | mMOLS Cl/L C.S.F. WATER | | $(Cl)_F{}^*/(Cl)P^*$ | | mMOLS UREA/L C.S.F. WATER | | $(UREA)_F/(UREA)_P$ | | mMOLS Na/L C.S.F. WATER | | $(Na)_F/(Na)_P$ | | $H_2O$/L PLASMA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Range | Av. | Range | Av. | Range | Av. | Range | Av. | Range | Av. | Range | Av. | Range | Av. |
| *cm.* | | | | | | | | | | | | | *grams* | |
| 3.5– 4.5 | 110–114(4) | 112 | 0.99–1.02 | 1.00 | 2.3–8.3(5) | 5.1 | 0.97–1.02 | 0.99 | | | | | | |
| 4.5– 5.0 | 106–124(4) | 115 | 1.01–1.08 | 1.03 | 6.1(2) | 6.1 | 1.02–1.03 | 1.03 | 108–131(3) | 126 | 0.98–1.05 | 1.01 | 977–982(2) | 980 |
| 5.0– 5.5 | 124(1) | 124 | 1.01 | 1.01 | 3.9–4.3(3) | 4.1 | 0.97–0.99 | 0.98 | 116–133(4) | 125 | 0.99–1.05 | 1.01 | | |
| 5.5– 6.0 | | | | | 5.8(1) | 5.8 | 0.82 | 0.82 | 119(1) | 119 | 1.10 | 1.10 | | |
| 6.0– 7.0 | 113–117(3) | 115 | 1.12–1.20 | 1.17 | 2.4– 3.9(6) | 3.5 | 0.78–0.91 | 0.85 | 120(1) | 120 | 1.09 | 1.09 | 980–985(4) | 982 |
| 7.0–10.0 | 110–115(3) | 113 | 1.19–1.24 | 1.22 | | | | | 108–133(3) | 125 | 1.0 –1.15 | 1.09 | 972–982(4) | 978 |
| 10.0–15.0 | 109–139(10) | 122 | 1.14–1.36 | 1.29 | 3.0–13.0(10) | 6.7 | 0.62–0.84 | 0.76 | 120–132(4) | 125 | 1.09–1.27 | 1.19 | 977–981(3) | 978 |
| 15.0–20.0 | 108–118(8) | 112 | 1.23–1.35 | 1.29 | 3.7– 5.3(7) | 4.4 | 0.74–0.86 | 0.81 | 122(1) | 122 | 1.07 | 1.07 | 962–976(7) | 970 |
| 20.0–30.0 | | | | | | | | | 102–167(5) | 130 | 1.08–1.22 | 1.13 | | |

quantity of plasma available in the smaller fetuses, collodion sacs, impermeable to protein, of only about 0.5 cc. capacity were used. Equilibrium was reached within 24 hours. Chloride and sodium distribution ratios were determined between plasma and its dialysate and the quantity of freely diffusible sodium calculated from these data.

The water content of plasma and cerebrospinal fluid was found by drying samples at 100° to 110°C. and noting the loss in weight.

*Results.* The variation in water content of blood plasma with fetal age is noted in table 1. There was an apparently appreciable decrease in the concentration of plasma water with increase in fetal age. No change could be measured in the concentration of cerebrospinal fluid water with fetal age and consequently an average value of 990 grams water per liter cerebrospinal fluid has been used for all ages in calculating the concentration of substances in the cerebrospinal fluid water.

The excess sodium of the plasma over the calculated concentration of ionized base (per cent of total sodium) averaged 7.5 per cent for embryos of C. R. length under 7.0 cm., 8.4 per cent for fetuses between 11 and 15 cm. and 8.6 per cent for fetuses from 20.0 to 22.0 cm.

Table 1 gives the results of chloride, urea and sodium determinations on the cerebrospinal fluid of the fetal pigs and the distribution ratios of these substances between cerebrospinal fluid and plasma water. All of these substances had a distribution ratio of one or approximately one up to a C. R. length of 5.0 or 5.5 cm. Between the C. R. lengths of 5.5 and 6.0 cm. these distribution ratios between cerebrospinal fluid and plasma became different from one; Na and Cl ratios increased, urea ratios decreased. No difference was found between the slaughter house material and that obtained by Caesarian section.

Discussion. The Donnan equilibrium ratio between fetal blood plasma and cerebrospinal fluid is not accurately known. It can be predicted with confidence, however, to be nearer unity than in the adult because of the lower protein content of fetal plasma and the higher protein content of fetal cerebrospinal fluid. Up to a C. R. length of 5.0 cm. or an intrauterine age of 40 days, that is, approximately for the first third of the gestation period, the cerebrospinal fluid of the fetal pig as judged by the distribution of Cl, Na and urea is in equilibrium with the plasma. The only energy necessary for formation of the fluid will consequently be supplied by the heart and the fluid can be considered an ultrafiltrate of the plasma.

In the period C. R. length approximately 5.0 to 6.0 cm. or intrauterine age 40 to 43 days (11), Cl, Na and urea attain distribution ratios like those frequently described for adult animals. These substances in the cerebrospinal fluid of the older fetuses are so far removed from equilibrium with the plasma that more energy than supplied by the heart must be used in formation of the fluid. In this later stage, consequently, ultrafiltration is not an adequate explanation of the formation of the fluid

(4, 5). The extra energy must be supplied by the principal source of the cerebrospinal fluid, the chorioid plexus, and the fluid is to be considered a secretion of the chorioid plexus.

The ability to demonstrate that components of fetal cerebrospinal fluid change from equilibrium with the plasma to concentrations definitely removed from equilibrium appears to support the view that the deviations from equlibrium which have been noted in adult animals are real, are not due to errors of determination and that the cerebrospinal fluid of the adult is to be considered a secretion.

SUMMARY

Distribution ratios of Cl, Na and urea between cerebrospinal fluid and blood plasma have been determined during fetal life in the pig. Up to a C. R. length of 5.0 cm. (intrauterine age 40 days) these substances in the cerebrospinal fluid are in equilibrium with the plasma. In fetuses of C. R. length 6.0 cm. (intrauterine age 43 days) or greater these substances are no longer at equilibrium. In the growth interval between C. R. lengths of 5.0 and 6.0 cm. (3 days) cerebrospinal fluid, consequently, is believed to change from an ultrafiltrate to a secretion.

I am grateful to Dr. Hugh C. McPhee, Chief of Bureau of Animal Industry, United States Department of Agriculture, for his coöperation in providing me with pregnant sows for Caesarian section at the Experimental Station in Beltsville. I wish also to acknowledge the assistance and interest shown in this work by Dr. G. L. Streeter and Dr. C. G. Hartman, of the Carnegie Embryological Laboratory. The experience of the latter in operative procedures on the reproductive organs has been of great help. Finally, I wish to express my indebtedness to the Wm. Schluderberg-T. J. Kurdle Company for their generous supply of slaughter house material.

REFERENCES

(1) Ball, E. G. and J. F. Sadusk, Jr. J. Biol. Chem. **113:** 661, 1936.
(2) Beattie, F. Biochem. J. **22:** 711, 1928.
(3) Butler, A. M. and E. Tuthill. J. Biol. Chem. **93:** 171, 1931.
(4) Flexner, L. B. Physiol. Reviews **14:** 161, 1934.
(5) Flexner, L. B. J. Biol. Chem. **121:** 615, 1937.
(6) Flexner, L. B. and I. Gersh. Carnegie Inst. Washington Pub. no. 479, Contrib. Embryol. no. 157, vol. 26, 1937.
(7) Hastings, A. B., H. A. Salvesen, J. Sendroy, Jr. and D. D. Van Slyke. J. Gen. Physiol. **8:** 701, 1927.
(8) Karr, W. G. J. Lab. and Clin. Med. **9:** 329, 1924.
(9) Looney, J. M. J. Biol. Chem. **88:** 189, 1930.
(10) Merritt, H. H. and F. Fremont-Smith. 1937. The cerebrospinal fluid. Philadelphia and London.
(11) Warwick, B. L. J. Morph. **46:** 59, 1928.
(12) Wilson, D. W. and E. G. Ball. J. Biol. Chem. **79:** 221, 1928.

# SALIVARY CONDITIONING IN ATROPINIZED DOGS

GLEN FINCH[1]

*From the Pavlovian Laboratory, Phipps Psychiatric Institute, Johns Hopkins University School of Medicine*

Received for publication July 7, 1938

I. In recent years several investigations have been concerned with the problem of defininining the locus of conditional response formation. Some of these studies have attempted to isolate the essential functional components of the conditioning process, while others have attempted to delineate anatomically the structural prerequisites of conditioning. Both types of study have yielded fruitful but not wholly univocal results. The present study was undertaken to re-examine one moot point, namely: *Can the conditioning process occur during the absence of the conditional response?*

Evidence bearing on this problem has been furnished by the work of Crisler (2), Harlow and Stagner (4), and Gantt and Light (5).

Crisler was able to establish conditional salivary responses to morphine injection in dogs whose salivation was suppressed by atropinization. The adequacy of his control of the long time effects of atropine injection might be questioned. As noted by Crisler, Mulinos and Lieb (6) had reported the formation of conditional salivary responses to atropine injection alone, and Doeve (3) had observed a "paradoxical" secretion of saliva after repeated atropinization, a phenomenon also noted by Arima (1). Crisler, unable to confirm these observations with two dogs who were given daily atropine injections for 30 days, concluded that "if such a conditioned reflex is possible its appearance seems to be too slow to complicate our results." Crisler does not further support this conclusion since the procedure followed during the morphine-atropine training was to give the animals the atropine injections 10 to 15 minutes *before* they were placed in the observation apparatus. Under these conditions it was impossible for Crisler to observe and measure such salivation as may have occurred in response to the atropine injection alone.

Harlow and Stagner subjected curarized animals to CR training. They

[1] Yale Laboratories of Primate Biology. This investigation was conducted while the writer was a National Research Council Fellow, Division of the Biological Sciences. Grateful acknowledgment is made to Dr. W. H. Gantt for his counsel throughout the experiment.

were able to demonstrate conditional smooth muscle (not paralyzed by curare) responses but failed to demonstrate conditional striate muscle (paralyzed by curare) responses in the animals after recovery from curarization. Following the same technique, CR's were readily established in normal animals. Harlow and Stagner state as one of the *implications* of these results that "conditioned reflexes are only possible if a response is made, and do not result merely from stimuli presented simultaneously."

Gantt and Light demonstrated conditional hind limb flexion responses in dogs after recovery from limb paralysis (induced by crushing spinal motor tracts) even though the unconditional stimulus (shock to paw) was applied only when paralysis prevented the flexion response from occurring. They conclude from this result that "in order to form the simple conditioned reflex, execution of the act is not required. Whether execution speeds up the elaboration, we are unable to say, as our experiments made no comparisons, but the difference cannot be great, as we obtained stable reflexes, lasting for weeks after the training period without reënforcement by the unconditioned stimulus (pain)."

II. Crisler's failure to confirm the observations of Mulinos and Lieb, of Doeve, and of Arima—observations seemingly substantially verified—seems to demand additional experimentation. Parotid fistulae were prepared in two dogs. (Crisler's dogs had submaxillary fistulae preparations. In several other respects the present technique departed from Crisler's.) After the animals had fully recovered from the fistulae operations, they were placed in the usual CR apparatus (described elsewhere (7)) and daily preliminary observations were begun. These preliminary observations, which serve as comparison and control for the later critical observations, consisted of 10 daily 30 minute measurements of parotid secretion with subjects held in apparatus but not otherwise stimulated; and then 5 daily 30 minute parotid secretion measurements with subjects given 1 ml. subcutaneous injections of normal saline solution 15 minutes after being placed in apparatus.

After completion of these preliminary observations, injections of 1 ml. aqueous solution of 10 mgm. atropine sulfate were substituted for the saline solution injections. This technique was continued with animal 1 for 19 days and with number 2 for 12 days. Figures 1 and 2 present the results for animals 1 and 2 respectively. Each of the points on the curves represents the volume of saliva secreted from the parotid gland during the first 15 minutes after the animal was placed in the apparatus. It can be observed from figures 1 and 2 that *1*, both animals secreted comparatively small volumes of saliva during the preliminary "spontaneous" and saline injection periods; *2*, the volume of secretion from each animal rose quite rapidly after the substitution of atropine for saline, and *3*, there is great daily variability in the quantity of saliva secreted.

While these results confirm the before-mentioned observations of Mulinos and Lieb, of Doeve, and of Arima, they should not be interpreted as invalidating or as necessarily contradicting Crisler's observations. In fact, the great differences in volume of secretion reported by Crisler (maximum about 25 ml. in 30 min.) and of the present experiment (less than 1 ml. in 15 min.) indicates that Crisler's results cannot be attributable to the action of atropine alone.

While too much emphasis should not be placed on the possibility that morphine-conditioned salivary responses in atropinized dogs may depend upon the conditioning of some correlative process (such as nausea), it seems desirable to determine whether it is possible when salivation is suppressed by atropine to establish conditional salivation using the more usual Pavlovian technique.

III. Animal 3 was subjected to 100 training trials (through 10 days) according to the following routine: 1, Subcutaneous injection of 1 cc.

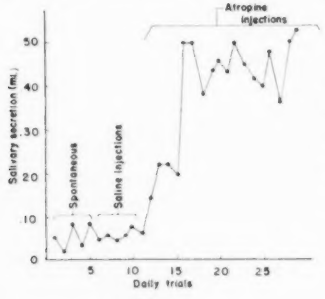



Fig. 1

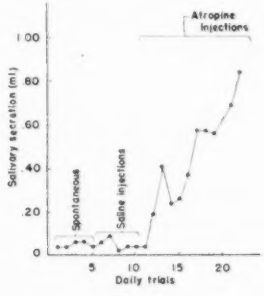



Fig. 2

Fig. 1. Salivary secretion during daily 15-minute test periods, animal 1
Fig. 2. Salivary secretion during daily 15-minute test periods, animal 2

aqueous solution of 10 mgm. atropine sulfate outside of the conditioning chamber; 2, 20 to 30 minutes later: animal placed in apparatus; 3, test of effect of atropine on salivation (biscuit placed in mouth and subsequent salivation measured); 4, if *no* salivation occurred, training proceeded; if animal salivated, beginning of training was delayed 5 minutes or until no salivation was elicited by biscuit; 5, 1st trial presented: bell rung 15 seconds, one dog biscuit presented 10 seconds after beginning of bell; 6, 2nd trial presented 2 minutes after beginning of 1st trial, and so on through 10 trials. Through the 100 training trials, no measurable quantity of saliva was secreted. It will be noted that the temporal order of stimuli was kept regular (CS presented at 2 min. intervals); thus the temporal interval may have acted as a conditioning stimulus as well as the sound of the bell.

At the conclusion of the 100 training trials, the strength of conditioning was tested. Using the same procedure but omitting both the atropine

injection and the unconditional stimuli (food presentation) 5 conditional stimuli were presented. The salivary secretion was measured during each 15 seconds the bell was ringing and during the 105 seconds between ringings. The results are presented in figure 3. Each point of the curve presents the volume of saliva secreted during each 15 second bell presentation less 15/105 of the volume secreted during the subsequent 105 seconds. This difference is presented rather than the gross volume secreted during the 15 second bell presentations in order to correct for any heightening of secretion which might be due to the atropine injections. Thus if the animal secreted saliva in a regular flow, the CR is presented as O. Any acceleration of the rate of flow during the 15 second bell interval appears as a positive response. An acceleration of the rate of flow during the non-bell interval might reduce the CR to a negative value.

Examination of figure 3 shows that only the first of the five stimuli elicited a CR—if such it may be called. Inasmuch as the other 4 stimuli

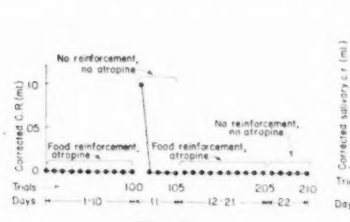



Fig. 3

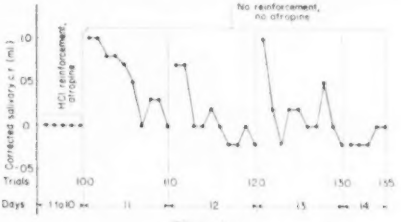



Fig. 4

Fig. 3. Corrected conditional salivary secretion (15-second test periods), animal 3.

Fig. 4. Corrected conditional salivary secretion (15-second test periods), animal 4.

elicited O responses, it may be that the positive response should be regarded as fortuitous since it is hardly conceivable that extinction of real conditioning should occur so quickly.

Throughout the 100 training trials the adequacy of the technique seemed questionable since the subject frequently failed to ingest the dog biscuits, which were presented as unconditional stimuli, and invariably exhibited symptoms of nausea (regurgitation, dyspnea) during the experimental sessions. Thus it is not at all surprising that the positive evidences of conditioning in this subject should be so slight.

Since these results were so inconclusive, dilute ($\frac{1}{2}$ per cent) hydrochloric acid injected into the mouth was substituted for food as unconditional stimulus for dogs 4 and 5, and training proceeded as for animal 3.

Animal number 4 was presented with 100 training trials during ten days before the strength of conditioning (i.e., trial without atropine and without unconditional stimulus) was tested. The strength of conditioning

was tested on the 11th, 12th, 13th and 14th days—each time through ten trials and without any intervening reinforcing trials. These results are presented in figure 4. During the first test-period (day 11), the CR, extinguished through ten trials, dropped from a maximal volume of 0.10 ml. to 0. On day 12, the CR had "spontaneously recovered," but persisted at a level of 0.07 ml. for only two trials. "Spontaneous recovery" occurred once more (day 13), but failed to occur on day 14.

Figure 5 presents the training procedure and data for animal 5. The strength of conditioning (measured with a single trial) after 5 days of training (50 trials) was 0. Training was discontinued after 10 days and the strength of the CR was tested with 10 trials daily for 2 days (days 11–12). As in the case of animal 4, the CR extinguished quickly, recovered its strength spontaneously and then extinguished more rapidly. An additional 50 trial training period (days 13–17) resulted in the establishing of a stronger CR which again persisted for only a short time. After

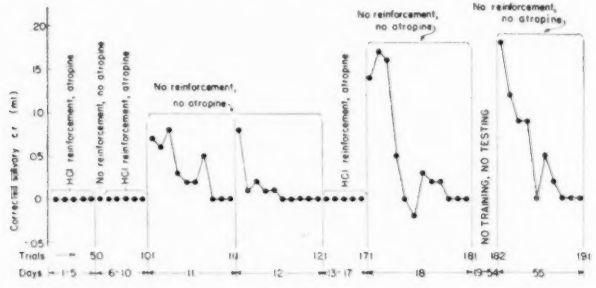



Fig. 5. Corrected conditional salivary secretion (15-second test periods), animal 5.

36 days had elapsed during which the animal was neither trained nor tested the CR had recovered its maximal strength.

These results (animals 4 and 5) demonstrate that conditioning, extinction, and spontaneous recovery of the salivary response follow much the same course in the atropinized dog as in the normal animal. Apparently the specific component, salivary gland response, is not a necessary prerequisite of salivary conditioning. These data confirm the findings of Gantt and Light and of Crisler.

## CONCLUSIONS

1. Daily subcutaneous injections of 10 mgm. atropine sulphate (2 dogs) resulted in a progressive heightening of the pre-injection parotid salivary secretion.

2. Conditional salivary responses were not established in one atropinized dog during training when food was presented as the unconditional stimulus. This failure may be attributed to the animal's refusal to ingest the food.

3. Conditional salivary responses were readily established in two atropinized dogs when dilute hydrochloric acid was injected in the mouth as the unconditional stimulus. Conditioning thus established underwent experimental extinction and exhibited "spontaneous recovery."

## REFERENCES

(1) ARIMA, H. Arch. exper. Path. u. Pharmakol. **83:** 157, 1918.
(2) CRISLER, G. This Journal **94:** 553, 1930.
(3) DOEVE, W. C. A. Ztschr. f. Tiermed. **18:** 77, 1914.
(4) HARLOW, H. F. AND R. STAGNER. J. Exper. Psychol. **16:** 283, 1933.
(5) LIGHT, J. S. AND W. H. GANTT. J. Comp. Psychol. **21:** 19, 1936.
(6) MULINOS, M. G. AND C. C. LIEB. This Journal **90:** 456, 1929.
(7) WOLFF, H. G. AND W. H. GANTT. Arch. Neurol. and Psychiat. **33:** 1030, 1935.

# ANTAGONISM BETWEEN POSTERIOR PITUITARY SECRETION AND ACETYLCHOLINE[1]

H. NECHELES AND F. NEUWELT

*From the Department of Gastro-Intestinal Research, Michael Reese Hospital, Chicago*

Received for publication May 16, 1938

The old theory of a relationship between the pituitary body and peptic ulcer was recently revived by Harvey Cushing (1). E. C. Dodds and collaborators (2) have produced gastritis and acute perforating ulcers in various animals by oral and parenteral administration of extracts of the posterior lobe of the pituitary gland. It has been found in our laboratory that acetylcholine (a.c.) produces vasoconstriction in the vessels of the human stomach and in the rats' and dogs' stomachs (3). A theory as to the genesis of peptic ulcer was developed, based on these observations and in correlation with certain anatomical findings, namely, the distribution of end arteries and vagal fibers in the human stomach (4). The thought in mind which prompted the present work was the possibility of a change in the activity of esterase following administration of pituitary extracts, which, in the light of our findings (3) might explain the occurrence of peptic ulcers observed by Dodds and collaborators (2). In a previous study we have found the bioassay of a.c. in the dog to yield rather constant values (5); usually 1 gamma of a.c. is injected intravenously and the drop in blood pressure is measured. Various anesthetics (ether, chloretone, morphine, nembutal) were used, the latter one being usually employed. We were quite surprised to find (fig. 1) that following the injection of pituitrin[2] or pitressin[2], but not of pitocin[2], the anticipated fall in blood pressure to injection of a.c. did not occur and remained more or less completely inhibited for some length of time. Repeated experiments, employing different anesthetics, and varying the dosage of pitressin (0.001 to 1.0 ccm., i.e., 0.01 to 10 units) showed that this inhibition is constant, the extent and duration of the inhibition in the fall of blood pressure being dependent upon the dosage of pitressin employed. We investigated this pituitrin a.c. antagonism in 11 experiments and in each an

[1] Aided by the Oscar Aberle and Otto Baer Funds.

A preliminary report was presented at the Meeting of the American Physiological Society, Baltimore, 1938.

[2] We wish to acknowledge the generous gift of these substances from Parke, Davis and Company.

adequate dose of pituitrin resulted in an inhibited a.c. blood pressure response. The reaction of dogs to pitressin is extremely variable; in one dog, e.g., 0.001 ccm. of pitressin caused a 60 per cent inhibition of the a.c. response for 1½ minute, and control values returned after 5 minutes. In another dog 0.1 ccm. was necessary to effect the same change. In another experiment 1 ccm. (10 pressor units) of pitressin completely abolished the response for 60 minutes. We found all gradations between these values

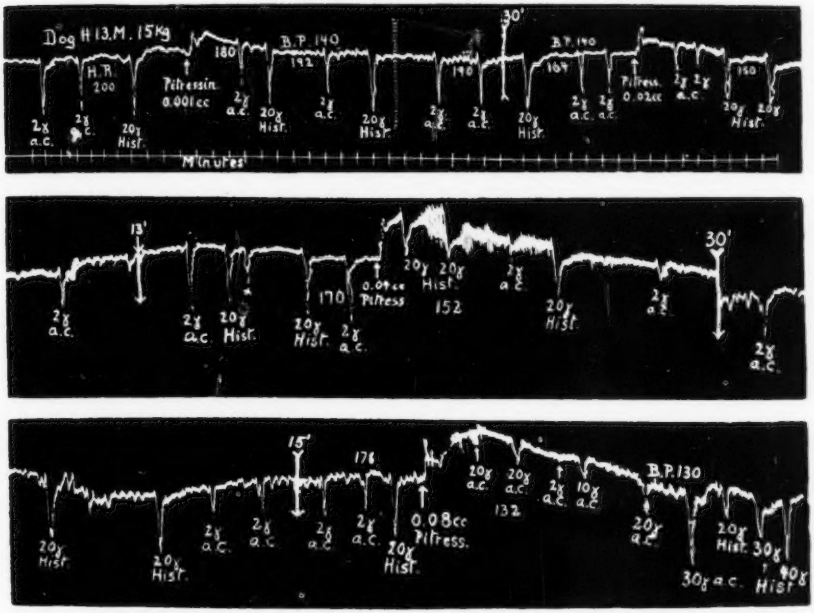



Fig. 1. Antagonism of pitressin to acetylcholine and histamine; 0.001 cc. (0.01 unit) of pitressin diminishes a.c. response, whereas 0.02 cc. (0.2 unit) of pitressin is necessary to cause slight inhibition of histamine response; 0.08 cc. (0.8 unit) of pitressin abolishes a.c. response completely whereas histamine response is only inhibited by this dose. Vertical arrows mark stopping of kymograph and numbers below the blood-pressure curve, the heart rate.

during the course of these experiments. This phenomenon was independent of changes of blood pressure in the dogs.

We soon found that pitressin does not owe its mode of action to any effects upon the a.c. esterase; that amount of pitressin which caused no change in blood pressure in the dog was incubated with a mixture of a.c. and blood for various periods of time and subsequent injections of such a mixture revealed a normal rate of destruction of a.c. (for details of methods see 5). The above experiment was then repeated, but this time

the blood used was obtained from a dog whose blood-pressure response to a.c. had been completely inhibited by a previous intravenous injection of pitressin. Again the rate of destruction of a.c. by the esterase was similar to that in controls. The in vitro incubation of pitressin and a.c. in saline without any blood being added, did not alter the effects of the a.c. in any way when this mixture was injected intravenously, i.e., such a mixture containing 1 gamma of a.c. plus that amount of pitressin which would cause no blood-pressure change in that particular dog, would cause a fall in blood pressure identical to that resulting from the injection of 1 gamma of a.c. only. These experiments proved that pitressin did not alter the blood-pressure response to a.c. and it certainly did not potentiate the action of a.c. esterase.

It is known that pitressin has a direct action on the smooth muscle cells of arterioles, capillaries and even venules, contracting them and leaving them contracted for some length of time; i.e., in a state of "static tone." The absence of vasodepressor effects of a.c. following pitressin we feel is due to some such mechanism, the a.c. being blocked from its effector organ, the myoneural junction. Histamine, in contrast to a.c., acts directly on the Rouget cells and results in dilatation and upon intravenous injection in proper dosage, causes an evanescent drop in blood pressure similar to the one evoked by a.c. We therefore proceeded to test the effects of histamine injected after pitressin (see fig. 1) and found that following the injection of pitressin, in doses larger than those required for a.c. inhibition alone, the histamine response was considerably diminished; it was never abolished completely as the a.c. response.

Dodds in studying the mode of ulcer production in his experimental animals found that the gastric secretory mechanism was strongly inhibited by pitressin (6). This observation led Dodds et al. to make studies on pancreatic secretion which they found inhibited following injection of posterior pituitary extracts; they also noted, as have others (Geiling, 7) that salivation was diminished by pituitrin injection. We have examined the problem of salivary secretion as influenced by pitressin during the course of our experiments above (fig. 2). The rate of salivary secretion was measured by means of a drop counter and the chorda tympani stimulated either electrically (Harvard inductorium) or by pilocarpine injected intravenously. Invariably, we obtained a diminution to a total cessation of salivary flow following the intravenous injection of varying quantities of pitressin. In connection with our findings, it is interesting to note that Gorke and Deloch (8) and Weir, Larson and Rowntree (9) observed that in patients with diabetes insipidus pitressin seemed to augment salivary secretion.

Lim and collaborators (10) have proven recently that faradic stimulation of the central vagi in viviperfused isolated heads of dogs (a one animal

preparation) causes the liberation of small, but demonstrable amounts of pituitrin resulting in an elevation of blood pressure of the body of the animal. We investigated this finding using our method of bioassay for a.c. In most of these experiments we have found that stimulation of the central vagi caused a diminution or abolition of the a.c. response, not only during the time of stimulation but even for some minutes after cessation of the stimulating current. We have failed to obtain this inhibition after cutting of the hypophyseal stalk (fig. 3) or severing of the brain stem (fig. 4)

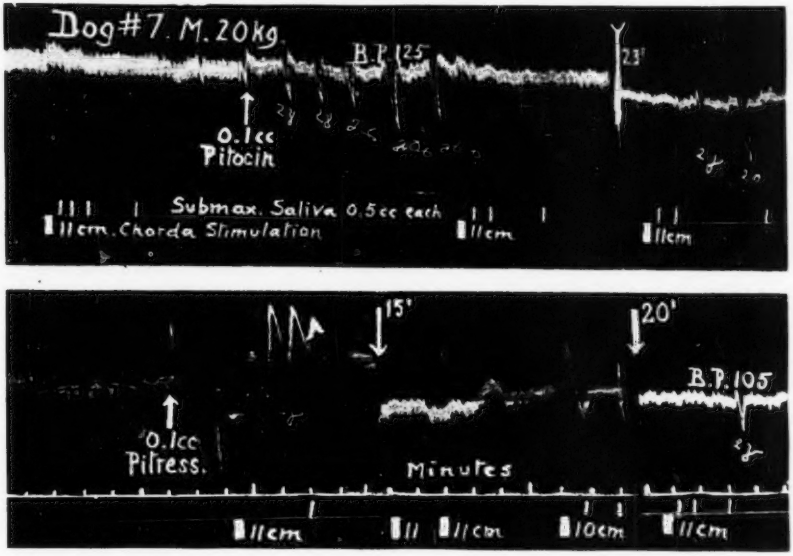



Fig. 2. Inhibition of salivary secretion (submaxillary) following intravenous injection of pitressin. Note: slight effect of pitocin probably due entirely to pitressin content. Latent effect of pitressin demonstrated by fact that first stimulation following pitressin causes secretion of 0.5 cc. of saliva whereas 20 minutes later the inhibition is complete. Control rate returned only after 50 minutes.

which rules out central vascular reflexes. Respiratory changes play no rôle since we have done control experiments with the thorax opened and artificial respiration; hypo- or hyper-ventilation of the lungs has no effect on this phenomenon.

During the course of this work we noted that pitressin modified the influence of peripheral vagus stimulation on cardiac inhibition. We note in figure 5 (representative of a number of experiments with similar results), that faradic stimulation of the peripheral ends of the cut vagi results in a marked slowing of the heart rate and a fall in blood pressure. Ten second

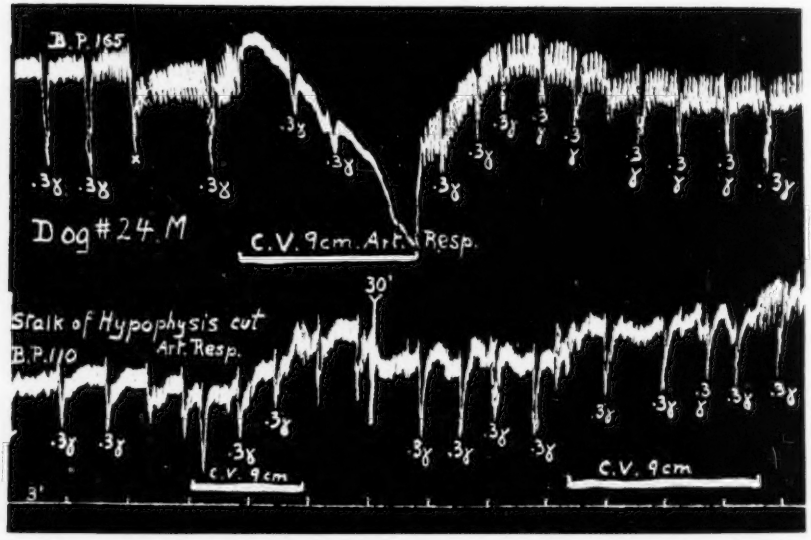



Fig. 3. Faradic stimulation of central vagi. Severing of the hypophyseal stalk abolishes a.c. inhibition following stimulation of central vagi. Note latent effects following control stimulation, i.e., a.c. response returns to normal only 16 minutes following cessation of stimulation. Artificial respiration was given during control stimulation of vagi and following cutting of hypophyseal stalk.

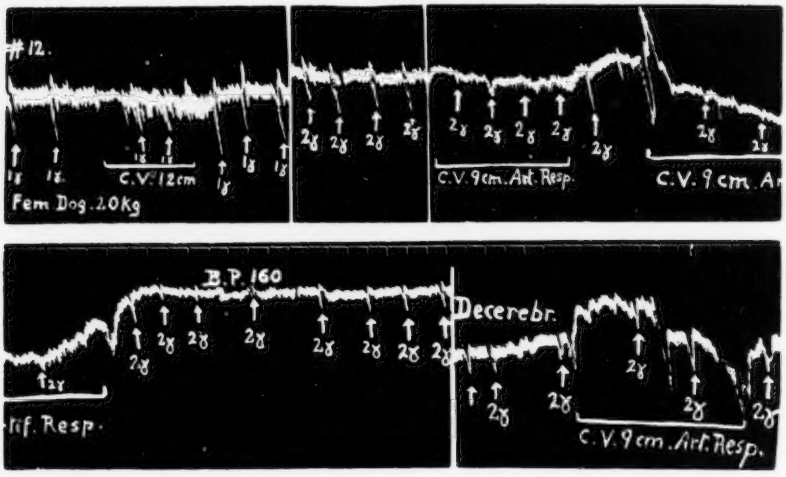



Fig. 4. Effects of section of brain stem on central vagus inhibition of a.c. response. Severing brain stem prevents effects on pituitary gland of stimulation of central vagi and thereby inhibition of vasodepressor effects of a.c. Note: artificial respiration plays no rôle in control inhibitions.

stimulation at 8 cm. coil distance (Harvard inductorium with 2 dry cells) slowed the heart rate from 180 to 28, 130 to 12, 176 to 24, 160 to 18 and 180 to 90 beats per minute. The injection of 1 unit of pitressin slowed the heart rate to 130 and stimulation of the vagi one minute after the injection caused the usual fall in blood pressure, but the heart rate dropped to zero. A minute later, on the other hand, vagus stimulation slowed the heart rate to 12. The subsequent injection of 2.5 units of pitressin caused no change in blood pressure, but vagal stimulation again reduced the heart rate to zero. This complete cardiac arrest may be explained by vagal inhibition inflicted upon a heart rendered ischemic and hypodynamic by the effects

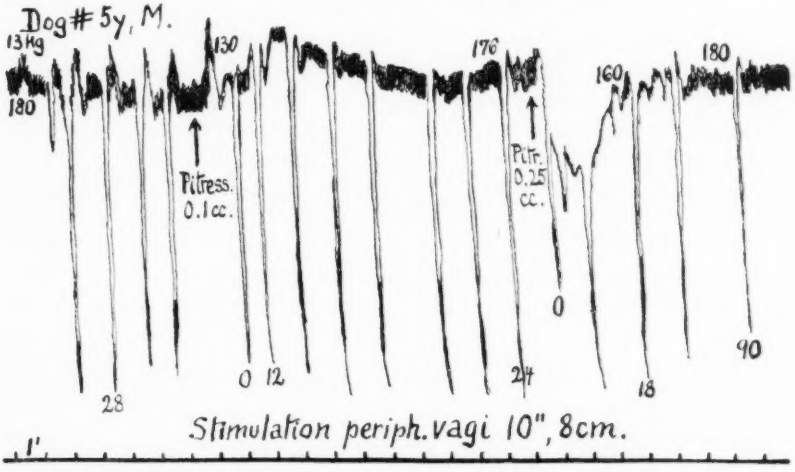



Fig. 5. Effect of stimulation of peripheral vagi on bloodpressure and heart rate. Numbers on top of graph represent control heart rates; numbers at the bottom of depressions of blood pressure represent heart rate during peripheral vagal stimulation.

of pitressin. Rogers (11) noted a similar phenomenon in decerebrated pigeons with the optic thalamus removed. He could cause a fatal cardiac inhibition in these birds by stimulating the cloaca, oviducts, etc., following the injection of pitressin.

Kolls and Geiling (12) have observed fluoroscopically and by means of teleoroentgenograms that administration of pitressin caused a dilatation of the heart; Resnick and Geiling (13) in a subsequent report studied by means of the electro-cardiograph the response of the heart to pitressin before and after the administration of atropine in normal unanesthetized dogs; they conclude from these studies that pitressin has an effect upon the heart through the cardio-inhibitor center as well as by its direct action

upon the heart muscle. More interesting, in view of later work by Melville, is the discussion by Resnick and Geiling of the similarity in action of pitressin and of anoxemia on the heart. Melville (14) in a series of publications in 1932 and 1933 proved that pitressin causes an extreme degree of coronary constriction so that cardiac output, rate and coronary arterial flow are very materially reduced. These observations explain why, following an adequate dose of pitressin, cardiac dilatation, cardiac arrest, and marked fall in blood pressure ensue.

## SUMMARY AND CONCLUSIONS

Small doses of pitressin or pituitrin injected intravenously inhibit more or less completely the vasodepressor effects of small doses of acetylcholine. Under adequate experimental conditions this may be used as a sensitive test for the presence of small amounts of pituitrin in the circulation, like those liberated by stimulation of the central vagi. Pitressin inhibits salivary secretion and affects the vagus response of the heart. The question may be raised whether the antagonism between a.c. and pituitrin represents a physiological mechanism. The effectiveness of such small amounts of pituitrin (or pitressin) as are liberated by central vagal stimulation suggests such a relationship.

## REFERENCES

(1) Cushing, H. Surg., Gynec. and Obstet. **55:** 1, 1932.
(2) Dodds, E. C., R. L. Noble, R. W. Scarff and P. C. Williams. Proc. Royal Soc. **123:** 22, 1937.
(3) Necheles, H., P. Levitsky, R. Kohn, M. Maskin and R. Frank. This Journal **110:** 330, 1936.
(4) Necheles, H. Am. J. Dig. Dis. and Nutr. **4:** 643, 1937.
(5) Ginsberg, R., R. Kohn and H. Necheles. Ibid. **4:** 154, 1937.
(6) Cutting, W. C., E. C. Dodds, R. L. Noble and P. C. Williams. Proc. Royal Soc. **123:** 27, 1937.
(7) Geiling, E. M. K. Physiol. Reviews **6:** 83, 1926.
(8) Gorke, H. and E. Deloch. Arch. f. Verdauungskrankh. **29:** 149, 1933.
(9) Weir, J. F., E. E. Larson and L. G. Rowntree. Arch. Int. Med. **29:** 306, 1922.
(10) Chang, H. C., K. F. Chia, C. H. Hsu and R. K. S. Lim. Chin. J. Physiol. **12:** 309, 1937.
(11) Rogers, F. T. This Journal **76:** 284, 1926.
(12) Kolls, A. C. and E. M. K. Geiling. J. Pharmacol. and Exper. Therap. **24:** 67, 1924.
(13) Resnick, W. H. and E. M. K. Geiling. J. Clin. Investigation **1:** 217, 1924–1925.
(14) Melville, K. I. Arch. Internation. de Pharmacodyn. et de Therap. **44:** 318, 1933.

# THE EFFECT OF CHLOROFORM ANESTHESIA ON SERUM AMYLASE AND LIVER ESTERASE[1]

F. A. CAJORI AND HARRY M. VARS

*From the Department of Physiological Chemistry and the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia*

Received for publication June 30, 1938

The administration of chloroform to animals, including man, has long been known to result occasionally in serious liver injury. Fatty infiltration, cell degeneration and necrosis are well recognized expressions of the liver injury caused by this agent. A less widely known effect of chloroform poisoning is the decrease in blood amylase found in dogs by Davis and Ross (1921) and also in cats by Zucker, Newburger and Berg (1932). These observations merit attention, for Somogyi (1934) has encountered low serum amylase in clinical cases involving liver injury such as hepatitis, cirrhosis and carcinoma of the liver. These findings have led Somogyi to believe that low blood amylase is associated solely with liver damage, and that a low serum amylase is of diagnostic value as an indication of altered liver function. Recently Rachmilewitz (1938) also has reported low serum amylase in hepatic disease.

The relation of serum amylase to the functional state of the pancreas has received much emphasis in recent years. A closer study of the correlation between *liver injury* and serum amylase is clearly desirable. As part of a study of the effect of certain anesthetics on liver metabolism we have studied the effects of chloroform anesthesia on the serum amylase and liver esterase of dogs. The experiments were planned so as to make them highly suitable for enzyme studies. The timing of anesthesia was carefully controlled. Chloroform volatilized with air was administered in one series of experiments and in a second series the chloroform was volatilized with oxygen. Histological and chemical examinations of the liver were made at stated periods after anesthesia. The details of the experimental procedure, including the post-anesthetic treatment of the animals, are given in the paper of Ravdin, Vars and Goldschmidt (1938). In the present report the results of serum amylase and liver esterase determinations are presented.

EXPERIMENTAL. For the amylase studies, blood samples were obtained just before beginning the anesthesia and afterwards usually at the time

[1] This work was aided by a grant from the Merck Fund for Surgical Research.

that the dog was sacrificed. In addition to the blood sample, a portion of the excised liver was obtained. The liver was passed through a meat grinder, dehydrated with acetone and the dried liver subsequently used for esterase determination. In view of the difficulties experienced by Davenport (1926) and Eadie (1927) in distinguishing between blood and liver amylase in liver tissue, no attempt was made to determine this enzyme in the liver itself.

Serum amylase was measured by incubating starch and serum for 10 to 20 minutes at 38° and determining the increase in reducing sugar resulting from starch digestion. The digestion mixtures consisted of 0.5 ml. of the blood serum being tested, 5 ml. of a 2 per cent soluble starch (Small, 1919) and 5 ml. of a 0.2 M phosphate buffer, pH 6.8, containing NaCl (0.04 M). After protein was removed by adding appropriate amounts of $ZnSO_4$ and NaOH, reducing sugar was determined in aliquots of the clear filtrate by the Hanes ferricyanide method (1929). Sugar was determined in a serum-starch mixture before and after incubation. The increase in reducing sugar in the incubated sample was the result of amylase activity. This serum amylase activity is expressed as the number of milligrams of reducing sugar, calculated as glucose, produced in one hour by 1 ml. of serum.

For the estimation of liver esterase, the acetone-dried liver was extracted at 38° for 3 hours with 0.025 M $NH_4OH$. The extract was acidified to methyl red with acetic acid. Acidification precipitated inactive protein (Baker and King, 1935), which was removed by centrifuging. Five-tenths milliliter of the filtrate, containing the esterase, was added to 50 ml. of 0.018 M methyl butyrate solution, the reaction adjusted to pH 7.0 and esterase activity determined at room temperature by the method of Knaffl-Lenz (1923). Esterase activity is expressed as ml. of 0.01 N acid produced from methyl butyrate in one hour by the extract of 1 gram of dry liver.

RESULTS. The amylase activity of the blood serum of the normal dogs (before anesthesia), though variable, usually was found to be between 40[2] and 70. An occasional serum was encountered with a high amylase content and also a few serums had an amylase value below 40.

After the administration of chloroform there was a decrease in the serum amylase and this occurred whether the initial value was high or low. In only three animals of the more than 50 which were studied, did we fail to find a decrease in serum amylase five hours or more after chloroform anesthesia (table 2).

[2] As explained above this means that, on the basis of the observed initial hydrolysis rate, 1 ml. of serum would produce 40 mgm. of reducing sugar, calculated as glucose, from starch in 1 hour at 38°. Dog serum has 20 to 25 times the amylase activity of human serum.

The decrease in serum amylase was greatest in the group of animals whose livers had suffered severe damage following chloroform anesthesia and in which marked changes in liver glycogen and fat had occurred. It will be seen in table 1 that an average decrease in serum amylase of 45 per cent was found in the dogs whose livers revealed evidence of necrosis when examined histologically. The decrease in serum amylase was also marked (average 38 per cent) in the group of animals with fatty infiltration of the liver, but no necrosis. Of 21 animals whose livers had undergone no visible changes following chloroform, 12 had a decrease in serum amylase of 20 per cent or more. Consistent changes in glycogen and fatty acids were also observed. The animals with fatty infiltration or necrosis of the liver had, on an average, a low liver glycogen and high liver fat. Some of the animals, especially those with extensive necrosis, developed jaundice after chloroform anesthesia and it might be presumed that the

TABLE 1

*Summary of changes in serum amylase following chloroform anesthesia as related to pathological changes in the liver and to liver glycogen and lipids*

| NUMBER OF EXPERIMENTS | BEFORE ANESTHESIA AVERAGE SERUM AMYLASE | AFTER ANESTHESIA | | | | |
|---|---|---|---|---|---|---|
| | | Histological appearance of the liver* | Average serum amylase | Percentage decrease in serum amylase | Average liver glycogen† | Average liver fatty acids† |
| 21 | 51 | Normal | 42 | 17 | 10.5 | 15.5 |
| 15 | 63 | Fatty infiltration | 39 | 38 | 9.6 | 18.5 |
| 5 | 62 | Slight necrosis | 35 | 44 | 5.5 | 18.2 |
| 15 | 52 | Extensive necrosis | 28 | 46 | 3.7 | 21.6 |

* The histological examinations were made by Dr. I. S. Ravdin.

† Per cent of dry weight.

low serum amylase observed at this time was the result of inhibition of the enzyme by bile. That this was not the case was shown by adding dog's bile to normal serum. No change in amylolytic activity of the serum occurred as the result of adding bile to it.

The amount of decrease in serum amylase was found to depend on the time that elapsed following administration of the anesthetic. The average decrease in serum amylase was greatest 24 to 48 hours after anesthesia. The decrease in serum amylase, however, was not as great when the after period was lengthened to 72 hours—a time at which liver regeneration is usually taking place. In blood samples obtained immediately following cessation of the anesthetic, or five hours later, changes in blood amylase were less pronounced and occasionally were absent.

Another factor which was found to influence the serum amylase was the length of time during which chloroform was administered. The average

decrease in amylase was decidedly less following a one-half hour anesthesia than it was following an hour of anesthesia (table 2). It is of interest to note that liver necrosis was not observed in any of the animals exposed to chloroform for the shorter time.

A greater incidence of liver necrosis was observed by Ravdin, Vars and Goldschmidt (1938) in the group of animals receiving chloroform volatilized with air than was found in animals receiving oxygen with chloroform. Here also the average serum amylase decrease (table 2) was somewhat greater, in the dogs receiving chloroform volatilized with air than in those of the chloroform-oxygen series.

Our results may be summarized in the statement that when experimental conditions were such as to produce excessive liver injury, maximum changes

TABLE 2

*The effect on serum amylase of the duration of anesthesia, the length of the after-period and the use of oxygen with chloroform or air with chloroform*

| DURATION OF ANESTHESIA | LENGTH OF AFTER-PERIOD | CHLOROFORM AND OXYGEN | | CHLOROFORM AND AIR | |
|---|---|---|---|---|---|
| | | Number of experiments | Average percentage decrease of serum amylase | Number of experiments | Average percentage decrease of serum amylase |
| *hours* | *hours* | | | | |
| 1 | 0 | 4 | −3* | 4 | 12 |
| 1 | 5 | 2 | 18 | 2 | 14 |
| 1 | 24 | 4 | 40 | 6 | 48 |
| 1 | 48 | 7 | 39 | 7 | 48 |
| 0.5 | 48 | 8† | 20 | 9 | 27 |
| 1 | 72 | 5‡ | 32 | 5 | 35 |

* An increase.

† Two animals in this group showed no decrease.

‡ One animal in this group showed no decrease.

in blood amylase were found. On the other hand, when the liver was afforded some protection, by a shorter period of anesthesia or by administering oxygen with the chloroform, the decrease in amylase was less. The relationship between damage of the liver and the level of blood amylase is clearly demonstrated in these experiments.

The close correlation between liver injury as revealed histologically and the accompanying change in serum amylase makes it probable that the low serum amylase of these dogs was the result of changes in the liver induced by chloroform. The liver is the organ showing the most marked pathological change in chloroform poisoning. If changes occur in other organs the histological alterations are usually not extreme (Howland and Richards, 1909; Whipple and Sperry, 1909).

The origin of serum amylase is not known. That part of it may be

of pancreatic origin, at least under conditions of pancreatic duct occlusion, cannot be doubted in view of the many observations on the effect on serum amylase of pancreatic duct obstruction. The persistence of amylase in the blood after pancreatectomy and the maintenance of a normal blood amylase in depancreatized dogs, maintained with insulin, leads to the conclusion that there must be sources of serum amylase other than the pancreas (Reid, Quigley and Myers, 1932-33). Experiments such as those reported in this paper are highly suggestive of a liver origin as well. If such a conclusion is valid, the fall in serum amylase is a delicate criterion of the toxic effect of chloroform on liver cells. It was found to occur frequently, when there was no visible histologic alteration of liver cells.

The possibility that dog serum amylase may arise from more than one place in the body suggests that more than one amylase may be present in blood. This problem was approached by studying the activity of serum

TABLE 3

*The relative activity of serum amylase at different hydrogen ion concentrations*

| pH | AMYLASE VALUE | RELATIVE ACTIVITY |
|---|---|---|
| | | *per cent* |
| 5.0 | 9.6 | 18 |
| 5.8 | 19.8 | 37 |
| 6.5 | 45.7 | 87 |
| 6.7 | 52.9 | 100 |
| 7.0 | 43.2 | 82 |
| 7.8-8.0 | 15.1 | 29 |

TABLE 4

*The esterase activity of liver tissue after chloroform anesthesia*

| NUMBER OF EXPERIMENTS | HISTOLOGICAL APPEARANCE OF THE LIVER | ESTERASE VALUE† |
|---|---|---|
| 2* | | 50 |
| 17 | Normal | 53 |
| 18 | Fatty infiltration | 48 |
| 5 | Mild necrosis | 55 |
| 17 | Severe necrosis | 26 |

* Unanesthetized.

† Ml. of 0.01 N acid produced per hour per gram of dry tissue.

amylase in phosphate buffers at different hydrogen ion concentrations. Eadie (1927) reported two pH optima for amylase in extracts of rat liver. He regarded one optimum as that of rat liver amylase and the other the pH optimum of an amylase present in the blood of the incompletely exsanguinated liver. On the other hand, Thompson and Friedman (1936) found no significant difference in the variations of amylase activity with change in pH between dog pancreatic extract, serum of a depancreatized dog and normal dog serum. It will be recalled that in man salivary and pancreatic amylase are identical as far as their behavior at different pHs is concerned.

In dogs' serum only one region of maximum amylase activity was found in the pH range studied (table 3). That was at pH 6.7–6.8. This result indicates that the amylases of dog serum, if indeed there is more than one, are so closely alike as to have the same pH optimum.

The results of the determinations of liver esterase are given in table 4. In addition to the esterase values determined in extracts of acetone-dried livers of the animals which had been anesthetized with chloroform, the livers of two normal (unanesthetized) dogs, were examined. They gave esterase values of 51 and 48 respectively.

It will be seen from the data summarized in the table that the average liver esterase value of the chloroformed animals did not deviate significantly from those of the two normal controls with the exception of the livers which were extensively necrotic. In the livers with severe necrosis the esterase activity was decreased markedly.

## SUMMARY

A decrease in serum amylase of dogs was found following chloroform anesthesia. The decrease was greatest in those animals whose livers contained areas of necrosis. A lowering of serum amylase was also observed in animals having livers with fatty infiltration and in animals having livers in which chloroform anesthesia had produced no visible liver injury.

The length of time of anesthesia, the administration of the chloroform volatilized with air, or with oxygen, and the time that was allowed to elapse after anesthesia were factors that influenced the level of blood amylase.

The optimum activity of dog serum amylase was limited to one range only of hydrogen ion concentration, pH 6.7 to pH 6.8.

Liver esterase was found to be decreased in livers that exhibited severe necrosis as a result of chloroform anesthesia.

## REFERENCES

BAKER, E. AND C. G. KING. J. Am. Chem. Soc. **57:** 358, 1935.
DAVENPORT, H. A. J. Biol. Chem. **70:** 625, 1926.
DAVIS, L. H. AND E. L. ROSS. This Journal **56:** 22, 1921.
EADIE, G. S. Biochem. J. **21:** 314, 1927.
HANES, C. S. Biochem. J. **23:** 99, 1929.
HOWLAND, G. H. AND A. N. RICHARDS. J. Exper. Med. **11:** 354, 1909.
KNAFFL-LENZ, E. Arch. exper. path. u. exper. Therap. **97:** 242, 1923.
RACHMILEWITZ, M. Am. J. Digest. Dis. **5:** 184, 1938.
RAVDIN, I. S., H. M. VARS AND S. GOLDSCHMIDT. J. Pharmacol. and Exper. Therap. (in press).
REID, E., J. P. QUIGLEY AND V. MYERS. J. Biol. Chem. **99:** 615, 1932-33.
SMALL, J. C. J. Am. Chem. Soc. **41:** 113, 1919.
SOMOGYI, M. Proc. exper. Biol. and Med. **32:** 564, 1934.
THOMPSON, W. B. AND I. FRIEDMAN. J. Gen. Physiol. **19:** 807, 1936.
WHIPPLE, G. H. AND J. A. SPERRY. Johns Hopkins Hosp. Bull. **20:** 278, 1909.
ZUCKER, T. F., P. G. NEWBURGER AND B. M. BERG. This Journal **102:** 209, 1932.

# THE ACTION OF EXCESS Na, Ca AND K ON THE CORONARY VESSELS[1]

L. N. KATZ AND E. LINDNER

*From the Cardiovasular Department, Michael Reese Hospital, Chicago*

Received for publication June 13, 1938

Recently, it has been shown that in circulatory disturbances such as shock a measurable change in the salt balance of the blood occurs in that an excess of K appears (1). Digitalis also tends to cause a similar disturbance in salt balance (2). On this account, we thought it desirable to investigate the action of excess K, Ca and Na on the coronary vessels. The method we have developed (3) of perfusing the denervated living coronary vessels in the dog with defibrinated dog's blood is particularly suitable since the changing extravascular factors which complicate the action in the beating heart are avoided when the ventricles are fibrillating and the perfusion of the coronaries is maintained at constant temperature and pressure. Under these circumstances changes in coronary flow are a direct measure of coronary vessel calibre.

METHOD. The preparation was identical with that previously described by us (3). The coronary perfusion was carried out via the aorta, and the coronary flow was measured by the outflow from the pulmonary artery. As in our previous experiments, 10 cc. of 10 per cent calcium gluconate (furnished by Sandoz Chemical Works) and 3 grams of glucose were added to the reservoir at the start; the total amount of defibrinated blood in the system was 3000 cc. Solutions of the chloride salts of K, Ca and Na were made up with distilled water (about 10 cc.) and, up to experiment 7, were injected into the coronary inflow tube. The concentration of the added salt in terms of cation added was determined on the basis of the cation added divided by the quantity of blood which passed the point of injection during the injection period, the last being equal to the product of the average rate of flow and the duration of the injection. In experiment 7 et seq. the salt solution was injected directly into the constant pressure inflow reservoir. To ensure a quick mixture of the desired concentration, the injection was made as the level in this reservoir was being raised. Once the mixture in this reservoir was made, the inflow into the reservoir was stopped until the reservoir was almost emptied when it was again filled

[1] Aided by the A. D. Nast Fund for Cardiac Research and Grants 237 and 283 of the Therapeutic Committee of the American Medical Association to L. N. K.

and kept at its former constant level. The concentration of the salt added was calculated in these experiments as the amount of cation added divided by the amount of blood in the reservoir to which it was added.

Discussion of results. The results are shown in tables 1, 2 and 3. Sodium chloride in all concentrations used consistently caused a significant increase in coronary flow indicating coronary vasodilatation (table 1). This was of short duration and occurred when the Na concentration was increased $1\frac{1}{5}$ to $2\frac{3}{4}$ times the normal concentration of Na present in defibrinated blood.

TABLE 1

*Effect of NaCl on coronary flow*†‡

| EXPERIMENT NUMBER | CONCENTRATION OF $Na^+$ IN BLOOD REACHING CORONARIES DURING INJECTION | CHANGE IN CORONARY FLOW | DURATION OF EFFECT | CONCENTRATION OF CATIONS IN BLOOD PREVIOUS TO INJECTION§ | | |
|---|---|---|---|---|---|---|
| | | | | $Na^+$ | $Ca^{++}$ | $K^+$ |
| | *mgm. per cent* | *per cent* | *minutes* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* |
| 2 | 200 | +25 | 2.5 | 168 | 17 | 220 |
| | 204 | +25 | 2 | 172 | 17 | 220 |
| | 252 | +27 | 2 | 185 | 61 | 259 |
| 3 | 223 | +48 | 2 | 168 | 21 | 229 |
| 5 | 456 | +93 | 2.5 | 227 | 35 | 252 |
| | 324 | +79 | 4.5 | 229 | 35 | 252 |
| *12 | 275 | +27 | 4 | 168 | 17 | 220 |
| | 382 | +32 | 2 | 222 | 48 | 246 |
| *13 | 275 | +80 | 4 | 168 | 17 | 220 |
| | 364 | +39 | 1.5 | 204 | 48 | 220 |
| *14 | 275 (−) | +135 | 2.5++ | 168 | 17 | 220 |
| Average | | +55.4 | 2.7 | | | |

Range of concentrations giving dilatation = 456 to 200 mgm. per cent.

* NaCl added to constant pressure inflow reservoir.

† Concentration of Na+ in whole blood (−fibrinogen) based on figures in Van Slyke and McLean (6) = 168 mgm. per cent.

‡ No effect noted on character of ventricular fibrillation.

§ Calculated on basis of quantity of blood in perfusing system assuming complete mixture of added salts with the entire perfusate.

Calcium chloride (with one exception, expt. 10) in all concentrations used also gave a significant increase in coronary flow indicating coronary vasodilatation (table 2).[2] The duration of the calcium effect was longer

[2] In the three injections in experiment 10, there were mixed responses with dilatation as the final stage. This experiment differed from the others in that saline had to be added to replenish the total volume of blood, thus leading to an unusually high sodium concentration in the perfusate.

than that with sodium, and the magnitude of the change was much greater. The dilatation (except in expt. 10) occurred with concentrations of Ca of from $1\frac{1}{3}$ to 24 times its normal concentration. With these larger quan-

TABLE 2

*Effect of $CaCl_2$ on coronary flow**

| EXPERIMENT NUMBER | CONCENTRATION OF $Ca^{++}$ IN BLOOD REACHING CORONARIES DURING INJECTION | CHANGE IN CORONARY FLOW | DURATION OF EFFECT | CONCENTRATION OF CATIONS IN BLOOD PREVIOUS TO INJECTION‡ | | | CHANGE IN CHARACTER OF VENTRICULAR CONTRACTIONS | EFFECT OF CHANGED VENTRICULAR ACTION ON CORONARY FLOW |
|---|---|---|---|---|---|---|---|---|
| | | | | $Na^{+}$ | $Ca^{++}$ | $K^{+}$ | | |
| | *mgm. per cent* | *per cent* | *minutes* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | | |
| 2 | 408 | +253 | 6.5+ | 185 | 24 | 259 | Fibrillation more vigorous | None |
| 3 | 72 | +54 | 1.5+ | 168 | 17 | 220 | No real change | |
| | 120 | +190 | 9.+ | 169 | 21 | 231 | No real change | |
| 4 | 149 | +140 | 6. | 168 | 17 | 250 | Synergic beating, then fibrillation | Increase with synergic beat, decrease with fibrillation |
| | 250 | +138 | 15.0+ | 168 | 30 | 250 | Fibrillation coarser then finer | Decrease with finer fibrillation |
| 5 | 139 | +58 | 6.5 | 227 | 17 | 249 | Standstill, then fibrillation | Increase with standstill |
| | 186 | +64 | 3.5 | 227 | 29 | 249 | No real change | |
| | 223 | +42 | 3 | 235 | 35 | 254 | No real change | |
| 8† | 183 | +49 | 6 | 168 | 17 | 257 | Fibrillation more vigorous | Decrease |
| 9† | 183 | +78 | 8 | 168 | 45 | 269 | No real change | |
| **10† | 22 | +8, −8, +20 | 5.+ | 287 | 17 | 220 | No real change | |
| | 28 | −15, +11 | 8.+ | 287 | 18 | 220 | No real change | |
| | 70 | −17, +25 | 11.+ | 287 | 21 | 221 | Fibrillation more vigorous | Increase |
| 11† | 27 | +28 | 5.5 | 168 | 17 | 220 | No real change | |
| | 71 | +23 | 6 | 169 | 22 | 221 | No real change | |
| Average..... | | +74 (−13) | 6.7+ | | | | | |

Range of concentrations giving dilatation = 408 to 22 mgm. per cent.

* Concentration of Ca++ in whole blood (−fibrinogen) based on figures in Van Slyke and McLean (6) = 5 mgm. per cent + 12 mgm. per cent (Ca gluconate added) = 17 mgm. per cent.

† $CaCl_2$ added to constant pressure inflow reservoir.

** Isotonic NaCl had to be added in this experiment at start because of lack of sufficient blood in reservoir.

‡ Calculated on basis of quantity of blood in perfusing system, assuming complete mixture of added salts with the entire perfusate.

tities of calcium the flow did not always return to its control level but remained elevated for some time.

The results with KCl were more complex. Examination of table 3

TABLE 3

*Effect of KCl on coronary flow*†

| EXPERIMENT NUMBER | CONCENTRATION OF $K^+$ IN BLOOD REACHING CORONARIES DURING INJECTION | CHANGE IN CORONARY FLOW | DURATION OF EFFECT | CONCENTRATION OF CATIONS IN BLOOD PREVIOUS TO INJECTION‡ | | | CHANGE IN CHARACTER OF VENTRICULAR CONTRACTIONS | EFFECT OF CHANGED VENTRICULAR ACTION ON CORONARY FLOW |
|---|---|---|---|---|---|---|---|---|
| | | | | $Na^+$ | $Ca^{++}$ | $K^+$ | | |
| | *mgm. per cent* | *per cent* | *minutes* | *mgm. per cent* | *mgm. per cent* | *mgm. per cent* | | |
| 2 | 229 | +42 | 2.5 | 181 | 17 | 220 | No real change | |
| | 348 | −80 | 8 | 181 | 24 | 238 | No real change | |
| 3 | 255 | +67 | 4.5 | 168 | 19 | 220 | No real change | |
| | 279 | +75 | 1.5+ | 168 | 19 | 222 | No real change | |
| 4 | 339 | −80 | 4.5+ | 168 | 17 | 225 | Fibrillation weaker, then standstill, later fibrillation | Decrease as fibrillation weakened, none with standstill, decrease with fibrillation |
| | 349 | −51 | 8 | 168 | 17 | 235 | Standstill | Decrease |
| | 529 | −53 | 7+ | 168 | 37 | 250 | Standstill | Decrease |
| | 371 | +120 | 6+ | 168 | 17 | 220 | No real change | |
| | 332 | +42 | 4.5 | 168 | 17 | 227 | Standstill, then fibrillation | Decrease with standstill, decrease with fibrillation |
| 5 | 384 | −57 | 9 | 228 | 17 | 242 | Standstill | Increase |
| | 367 | +42 | 3 | 228 | 40 | 249 | Standstill, then fibrillation, later synergic beating | Decrease with standstill, decrease with fibrillation, none with synergic beating |
| 7* | 515 | −96 | 46+ | 168 | 17 | 220 | Standstill | None |
| | 367 | −31 | 6 | 168 | 17 | 220 | Standstill, then fibrillation | Increase with standstill, none with fibrillation |
| 8* | 379 | −90 | 20.5 | 168 | 17 | 232 | Standstill, then fibrillation | None |
| 9* | 367 | +25 (−28) | 3, (15) | 168 | 17 | 220 | Standstill, then synergic beating, later fibrillation | None during standstill or synergic beating, increase with fibrillation |
| Average for increase | | +51.9 | 3.3+ | | | | | |
| Average for decrease | | −62.2 | 13.7+ | | | | | |

Range of concentrations giving constriction = 529 to 339 mgm. per cent.

Range of concentrations giving dilatation 371 to 229 mgm. per cent.

* KCl added to constant pressure inflow reservoir.

† Concentration of $K^+$ in normal whole (−fibrogen) based on figures from Van Slyke and McLean (6) = 220 mgm. per cent.

‡ Calculated in terms of cation on basis of quantity of blood in perfusing system, assuming complete mixture of added salts with the entire perfusate.

will show that when the K added had a concentration of from $1\frac{1}{2}\frac{1}{5}$ to $1\frac{2}{3}$ times the concentration of K present in defibrinated blood, an increased coronary flow occurred indicating coronary vasodilatation. With the presence of concentrations of from $1\frac{1}{2}$ to $2\frac{1}{2}$ times the concentration of K present in defibrinated blood, a decreased coronary flow occurred indicating coronary vasoconstriction. In other words, addition of K in amounts falling within the range of $\frac{1}{2}$ to $\frac{2}{3}$ the normal K content might produce either a dilatation or a constriction—even in the same preparation. In experiment 9 there was a diphasic response, consisting of dilatation for 3 minutes and then constriction for 15 minutes. The dilatation with potassium was relatively fleeting and occurred with smaller concentrations, whereas the constriction occurred with larger concentrations and was longer lasting. The duration of the constrictor action of K was longer than the dilatation action of Ca. Often the flow was found to remain below the control level for long periods of time. In some of our preliminary work, we found on several occasions that K in concentrations larger than those included in this table caused cessation of the coronary flow indicating complete coronary closure.

Sodium chloride injections had no apparent effect on the character of the ventricular fibrillation but both calcium and potassium altered it. Of the 15 tests with calcium, no significant changes in the character of the fibrillation occurred in 6; in 4, the fibrillation became more vigorous; in one a transient regular synergic beating of the ventricles appeared and in one, transient ventricular standstill occurred. Of the 16 tests with potassium, no significant changes occurred in 6, in 1 the fibrillations became finer and less vigorous, in 2, transient regular synergic beating of the ventricles appeared and in 10, ventricular standstill of varying duration occurred. These changes in the character of the beating of the ventricles afforded an opportunity to see whether these changes were associated with any consistent changes in the coronary flow. Inspection of tables 2 and 3 will show that no consistent correlation between flow and character of ventricular action occurred, indicating that in this preparation with the cavities of the heart open to atmospheric pressure, the action of the ventricles has no significant effect on the coronary flow.

Our results on the action of these salts on coronary flow are more consistent and probably more applicable to the reaction of the coronary blood vessels in the intact animal than the results of Cohen (4) on isolated surviving beef coronary rings suspended in oxygenated Ringer solution. Our results are not in accord with those on Langendorff preparations of the cat and rabbit heart obtained by Gruber and Roberts (5) who found that omission of K from Ringer's solution used as perfusate slowed coronary flow and that omission of Ca caused increased coronary flow. The differences between their results and ours are probably due to the differences in

perfusing media used. We believe that defibrinated blood approaches normal blood more closely than does Ringer's solution.[3]

The constriction of the coronary vessels with excess of K may be of significance in contributing to the circulatory failure noted in hemorrhage, shock and adrenal cortical insufficiency where recent work by Zwemer and Scudder (1) has shown the blood potassium level may rise to 50 to 100 per cent above normal values, particularly since the range of rise is of the order which in our preparation caused the marked coronary constriction. Such marked coronary constriction with K could lead to heart failure and thereby contribute to establishing the irreversible vicious cycle leading to death in these conditions.

The value of the intravenous use of Ca and Na salts as coronary dilators suggested by this study merits further observation. Potassium salts on the other hand, especially in large quantities, would appear from this study to be hazardous for intravenous use.

## SUMMARY

1. Langendorff preparations of dog hearts with ventricles fibrillating were perfused under constant pressure and constant temperature with defibrinated heparinized dog blood containing 10 cc. of 10 per cent calcium gluconate and 3 grams glucose. The chlorides of sodium, calcium and potassium dissolved in small amounts of distilled water were either injected directly into the coronary inflow tubing or added to the perfusion reservoir, and the effect on coronary flow noted.

2. Sodium and calcium produced a dilatation of the coronary vessels, the effect of the latter being longer in duration.

3. Potassium in dilute concentrations produced a brief dilatation; in stonger concentrations, a long-lasting constricton, at times almost to the point of complete coronary occlusion.

4. The significance of these results are briefly discussed.

We are indebted to the members of the department for assistance with these experiments.

## REFERENCES

(1) Zwemer, R. L. and J. Scudder. This Journal **119:** 427, 1937.
(2) Cattell, M. and H. Goodell. Science **86:** 106, 1937.
(3) Katz, L. N., E. Lindner, W. Weinstein, D. I. Abramson and K. Jochim. Arch. intern. Pharmaco. Dyn. (in press).
(4) Cohen, S. E. Arch. intern. Pharmaco. Dyn. **54:** 1, 1936.
(5) Gruber, C. M. and S. J. Roberts. This Journal **76:** 508, 1926.
(6) Van Slyke, D. D., H. Wu and F. C. McLean. J. Biol. Chem. **56:** 765, 1923.

[3] It is also possible that some of the effects in Gruber's and Roberts' experiments were secondary to the action of the cations on the character of the ventricular contractions.

# CHANGES IN THE SKIN TEMPERATURES OF THE EXTREMITIES PRODUCED BY CHANGES IN POSTURE

GRACE M. ROTH, MARVIN M. D. WILLIAMS AND CHARLES SHEARD

*From the Section on Clinical Physiology, and the Division of Physics and Biophysical Research, The Mayo Foundation, Rochester, Minnesota*

Received for publication June 13, 1938

In order to evaluate the effects produced by changes of posture in man on the peripheral supply of blood to the extremities, as evidenced by changes in skin temperatures, certain criteria must be met concerning the maintenance of constant environmental conditions, the permissible range of environmental temperatures and the metabolic state of the subject under test.

The investigations of Pickering and Hess (1933), Maddock and Coller (1933), Burton and Murlin (1935) and Sheard, Williams and Horton (1938) have shown that, on the one hand, great peripheral vasoconstriction exists in the extremities, as indicated by the low levels of the temperatures of the toes and fingers, in environmental temperatures of 17 to 20°C. (62.5 to 68°F.). On the other hand, great vasodilatation is present under environmental temperatures of 31 to 33°C. (88 to 91.5°F.), as is evidenced by the fact that the temperatures of the fingers and toes approximate the temperatures of the forehead and trunk. Because great vasoconstriction or vasodilatation of the peripheral vascular system of the extremities would, in turn, inhibit or mask the changes in skin temperature produced by changes in posture, the range of environmental temperatures to be employed lies between 23 and 30°C. (73.4 and 86°F.). In general, a room temperature of 25°C. (77°F.) and a relative humidity of 40 per cent are satisfactory.

The investigations should be made on subjects in the basal metabolic state because, under environmental temperatures ranging from 20 to 30°C. (68 to 86°F.) approximately, it has been shown by Sheard, Williams and Horton that, during the period of an hour of two after the ingestion of food, there is a rather rapid rise in the temperatures of the fingers and toes. Hence the rôle of the extremities as the regulators of the dissipation of heat from the body subsequent to an increase in the production of heat may predominate and, therefore, obscure or appreciably modify the effects attributable to changes of posture.

Another factor which may offset the effects of change in posture is the

presence of moisture on the extremities, commonly referred to as damp hands and feet, which occurs among some individuals in the range of environmental temperatures used in these investigations. In the event that sweating of the extremities occurs there will be a loss of heat by evaporation which will cause a reduction in the skin temperatures. Such a reduction in skin temperatures may annul or possibly reverse the effects caused by changes of posture.

Throughout these investigations, therefore, the effects of changes of posture on the peripheral blood supply to the extremities, as indicated by changes in the skin temperatures, have been determined on normal subjects in the basal state when placed under controlled optimal environmental conditions.

PROCEDURES. Data were obtained on fifteen normal men ranging in age from eighteen to thirty-six years. During the tests the subjects wore pajamas of light weight, so arranged that the arms and legs were well exposed. In general, the person under test, in the basal metabolic state, was placed in the horizontal position for a period of thirty minutes to an hour before any readings on the skin temperatures were made. The temperatures of the plantar surfaces of the first and third toes of both feet, the volar sides of the distal phalanges of the first and third fingers of the two hands, and of the forehead were measured by means of copper-constantan thermocouples. The thermocouples were fastened in position by narrow strips of adhesive tape and were left in situ during the period of time (usually several hours) consumed in the test. By means of switches, it was possible to put any desired thermocouple in the galvanometric circuit; the deflections of the galvanometer, converted into equivalent degrees centigrade, gave readings of the temperatures of the skin with an accuracy of 0.2°C.

RESULTS. *Effects produced by postural changes of the extremities only.* In a series of measurements of the temperatures of the fingers and toes of six subjects, lying horizontally on a bed in a room temperature of 25°C. (77°F.) a definite decrease in the temperatures of the fingers occurred when the arms were held perpendicular to the body. The arms were maintained in the upright position by means of a support for the fingers. The skin temperatures of the toes were unchanged or varied in a manner which indicated that correlation with the changes in the temperature of the fingers was not possible. The temperatures of the fingers returned to the level which they had previously maintained when the arms were again placed in the horizontal position.

In fourteen sets of observations the feet only were raised from the horizontal position and placed at an angle of 25 degrees. In all instances the skin temperatures of the toes decreased; the changes in the temperatures of the fingers were not significant. For example, there was a decrease of

about 4°C. in the temperatures of the toes when the feet only were elevated and, after the replacement of the legs horizontally, subsequent return of the temperatures of the feet to the value initially obtained in the horizontal position.

Seventeen sets of time-temperature relationships were determined for subjects who, lying in the horizontal position, lowered one leg over the side of the bed, the foot resting on a stool covered with a blanket. In all but two instances there was a definite increase in the temperatures of the toes of the dependent foot. In several instances in which the arm and leg of the same side of the body were lowered over the side of the bed but not allowed to come in contact with the floor, there was an increase in the skin temperature of the dependent extremities and a decrease in the temperatures of the toes and fingers of the other extremities maintained in the horizontal position.

*Effects produced by postural changes of the body.* In a set of twenty-two observations made on a group of subjects who changed from the horizontal to the sitting position it was found that the skin temperatures of the toes increased when the feet were pendent and returned to the level of values previously obtained when the subjects again assumed horizontal positions. In general, the skin temperatures of the fingers were higher when the subjects were in the sitting position.

In order to eliminate any cyclic effects which might occur by reason of the order of change of posture, measurements of temperature were made in a number of cases in which the sitting position was assumed initially, followed by a change to the horizontal position and, subsequently, a return to the sitting posture. In all instances, the skin temperatures of the toes and fingers were higher when the subjects were in the sitting position.

In fifteen of a series of twenty-three observations made on a group of subjects who changed posture from the horizontal to the upright position, the subject in each instance standing on a support covered with a blanket, the temperatures of the toes increased after the assumption of the upright position. In two instances, the temperatures of the toes remained unchanged and, in six instances, a slight decrease in temperature occurred when the posture was changed from the horizontal to the upright position. In general, the changes in the skin temperatures of the fingers paralleled those of the toes but were much more limited in range. In the few instances in which the temperatures of the toes decreased after the upright position was assumed, there was also a considerable fluctuation in the skin temperatures of the fingers, indicating a definite change in vasoconstriction of the extremities in order to prevent the loss of heat from the body occasioned by the change of environmental conditions.

Figure 1 shows the character of the changes in the skin temperatures of the toes produced by changes of posture from the horizontal to the upright

position. When the subject was horizontal on a bed in an environmental temperature of 25°C. (77°F.), the temperatures of the toes were approximately at room temperature for a period of about two hours. When the subject stood upright on a stool covered with a blanket, the temperatures of the toes rose during the course of an hour to approximately 30°C. (86°F.) and maintained this temperature for thirty minutes. On returning to the horizontal position, the temperatures of the toes decreased and assumed a value of 26 to 27°C. (78.8 to 80.6°F.), or approximately the temperature of the toes when the subject was in the initial horizontal position.

Objection may be raised to the results obtained regarding the changes in temperatures of the toes when the subjects were standing, for it is possible that the effects produced by changes of posture, as evidenced by changes in skin temperature, may be affected by pressure on the feet with an accom-

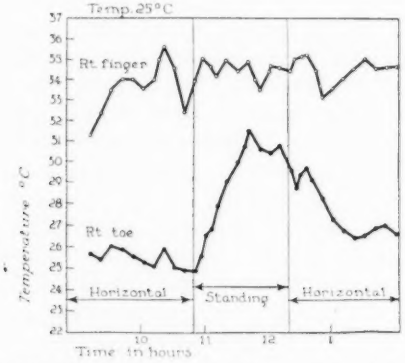



Fig. 1. Elevation of the skin temperature of the toes on voluntary change from the horizontal to the standing position, with a decrease of the skin temperature of the toes on the resumption of the horizontal posture.

panying cyanosis when the subject stands without movement of the extremities for a prolonged period of time. Also, the change of environment from the horizontal position to the sitting or upright posture, in which the dorsal side of the body is no longer in contact with the bed, may cause changes in the temperature of the extremities, particularly in the feet, by reason of the rôle which the extremities play in the fine adjustment or regulation of the dissipation of heat. The possibility of these complicating factors was obviated by the use of a tilt-table. When the subject was on the table tipped at an angle of 15 to 20 degrees, muscular effort was not required to maintain the position and it was not necessary for the feet to come in contact with any support. Hence any changes in the temperatures of the toes should be attributable primarily to physiologic causes. A series of twenty observations demonstrated that there was a definite in-

crease in the skin temperatures of the toes and, generally, of the fingers when the subjects were changed from the horizontal position to one at an angle of 15 or 20 degrees, the feet and hands being inclined downward. In no instance did the temperatures of the toes and fingers become less than those obtained with the subject in the horizontal position. Accompanying these changes in skin temperature there was an average increase in blood pressure of 8 mm. of mercury systolic and 13, diastolic, with an average increase in the heart rate of 18 beats per minute.

In several instances in which the subjects were tilted initially at an angle of 15 or 20 degrees, with the feet and hands inclined downward, for a period of an hour or more and, then, returned to the horizontal position, it was found that the temperatures of the fingers and toes were higher when the subjects were in the tilted position. A subsequent return to the original angle of tilt caused an increase in the temperatures of the toes and fingers.

COMMENT. These results are at variance with the findings of Youmans, Akeroyd and Frank (1935). They found the skin temperatures of the extremities to be several degrees higher among subjects who were placed in environmental temperatures of the same range as we have employed in our investigations. Of greater significance is the fact that they found a decrease in the temperatures of the toes of normal subjects in the upright position, whereas we have found an increase of temperature in the procedures we have followed. It might be possible to explain the disparity of results on the basis that their observations were made after the subjects had eaten a light lunch, whereas our observations were made on subjects in the basal metabolic state. In general, following the ingestion of food by a person in the basal metabolic state, in the horizontal position and in an environmental temperature of 25 to 28°C. (77 to 82.4°F.), there is a rise in the temperatures of the extremities so that, in the course of an hour or so, the temperatures of the forehead, trunk and extremities are approximately the same. This high level of temperatures of the extremities may be maintained for some time. These experimental observations may explain the relatively high temperatures of the extremities which were obtained by Youmans, Akeroyd and Frank, even in rooms at temperatures lower than 25°C. (77°F.). Furthermore, the period of high temperatures of the extremities subsequent to the ingestion of food is followed by a period of rather slowly decreasing temperatures. It seems probable, therefore, that the difference between our results and the findings of other observers may be explained, in part at least, by the fact that the rôle of the extremities in the control of the dissipation of heat from the body was predominant and obscured the effects produced by changes of posture.

By carefully controlling the periods of time, subsequent to the ingestion of food, at which changes of posture were made and by a comparison of the results with the cycle of changes in the temperature of the extremities

after the ingestion of food with the subject in a horizontal position, we have obtained the same type of changes in the skin temperatures of the toes and fingers as we have found among subjects in the basal metabolic state. The data in figure 2 were obtained from studies of the toes of a subject placed in an environment of 25°C. (77°F.) and a relative humidity of 40 per cent, prior to and subsequent to, the ingestion of food. The dotted line, labelled digestive cycle, is the time-temperature curve of the toes of the same person obtained on another occasion and under the same environmental conditions, the subject remaining in the horizontal position throughout the test. The effects of posture on the temperatures of the toes are of the same general character after the ingestion of food as when the subject is in the basal metabolic state.

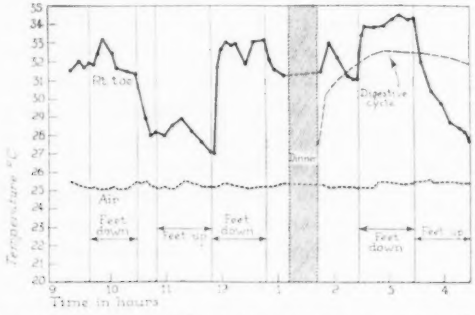



Fig. 2. Prior to the ingestion of food, the skin temperatures of the toes increase with the feet dependent and decrease with the feet elevated. Following the ingestion of food, the same type of change in skin temperatures of the toes is produced by postural changes of the feet as occurs when the subject is in the basal metabolic state.

The change in hydrostatic pressure, which is produced by the assumption of an upright posture, results in 1, a decrease in the velocity of the blood as shown by Bock, Dill and Edwards (1930); 2, an increase in the venous pressure in the lower extremities which is nearly equal to that of the hydrostatic pressure, as has been shown by many investigators, and 3, an average increase of about 4 per cent in the volume of the lower extremity as Grill (1937) demonstrated by means of the plethysmograph. These experimental results serve as a basis for the explanation of the changes in the skin temperatures of the extremities with the changes of posture which we have observed.

## SUMMARY

Observations of the effects of posture on the skin temperatures of the toes and fingers were made under environmental conditions which did not

produce either great vasoconstriction or great vasodilatation of the extremities but which permitted the fingers and toes to act as the fine adjustors in the control of the dissipation of heat from the body. Under environmental temperatures ranging from 23 to 30°C. (73.4 to 86°F.) with the subjects in the basal metabolic state and free from sweating of the extremities, the skin temperatures of the toes and fingers decreased when the extremities were elevated and increased when the extremities were pendent. When the posture of the body was changed from the horizontal, either voluntarily or when placed in an angular position by means of a tilt-table, the skin temperatures of the toes increased when the body was tilted with the feet downward and vice versa. These results probably are owing to changes in the peripheral circulation as a result of changes in hydrostatic pressure.

## REFERENCES

Bock, A. V., D. B. Dill and H. T. Edwards. J. Clin. Investigation **8:** 533, 1930.
Burton, A. C. and J. R. Murlin. J. Nutrition **9:** 281, 1935.
Grill, C. Acta med. Scand. **92:** 267, 1937.
Maddock, W. C. and F. A. Coller. This Journal **106:** 589, 1933.
Pickering, G. W. and W. Hess. Clin. Sc. **1:** 213, 1933.
Sheard, C., M. M. D. Williams and B. T. Horton. This Journal **119:** 403, 1937; Proc. Staff Meet. Mayo Clinic **13:** 13, 1938.
Youmans, J. B., J. H. Akeroyd, Jr. and H. Frank. J. Clin. Investigation **14:** 739, 1935.

# THE ABILITY OF THE DOG TO UTILIZE VITAMIN A FROM PLANT AND ANIMAL SOURCES

DOROTHY BRADFIELD AND MARGARET CAMMACK SMITH

*From the Department of Nutrition of the Arizona Agricultural Experiment Station*

Received for publication May 31, 1938

That dogs require vitamin A for normal growth and development and exhibit the symptoms which are characteristic of vitamin A deficiency when it is not present in adequate amounts in the ration has been shown by several investigators (1).

Although evidence is ample to show that the rat and herbivorous animals can utilize carotene, the plant precursor of vitamin A, as efficiently as vitamin A of animal origin, Rea and Drummond (2) on the basis of their experiments with cats have suggested that possibly carnivorus animals can not convert the vegetable precursor carotene to vitamin A. On the other hand Ralli (3) reported the presence of carotenase in the livers of some dogs receiving carotene but not in others. Frohring (4) detected no vitamin A in the livers of most of the puppies fed carotene for a period of three to twelve weeks but found a slight amount in the liver of a control dog receiving a high level of carotene (6500 units). Again, Busson and Simonnet (5) observed some storage of vitamin A in the livers of dogs previously fed carotene but they believed that there was a limit to the amount of carotene which the dog could utilize.

It is the purpose of this paper to present evidence to show the comparative efficiency of utilization by the dog of vitamin A in cod liver oil, and the vitamin A equivalent of its precursor, carotene, fed either as pure carotene in oil or as the plant food, carrots.

EXPERIMENTAL PROCEDURE. Twenty-seven young puppies, from 4 litters ranging from five to seven weeks of age, were used in the following experiments designed to compare the utilization of vitamin A obtained from animal and plant sources. The length of the experimental period was 14 to 22 weeks, during which time the pups received 47.5 grams of Morgan's modification of the Karr Cowgill (6) vitamin A deficient diet per kilogram of body weight.

The basal ration was supplemented with vitamin A obtained from cod liver oil, at daily levels of 20, 40, 100 or 200 U. S. P. units of vitamin A per 100 grams of body weight, or as the equivalent of 20 U. S. P. units of vitamin A in the form of a solution of carotene in oil, or in the natural form, carrots.

When the size of the litter permitted, one dog was placed upon each of the above levels, and one was kept as a negative control, receiving no vitamin A, comparisons being made within the litter so that the differences in nutritional history of the mother, exact age at beginning and end of experiments were excluded.

The cod liver oil used was obtained in one lot from Mead Johnson and Co. and contained 1800 U. S. P. units of vitamin A per gram. Carotene in oil, equivalent in vitamin A value to 7500 U. S. P. units per gram, was obtained from S. M. A. Corporation. The carrots used were of good grade and color, purchased on the open market. Their calculated equivalent in vitamin A units was the average figure of 50 U. S. P. units per gram, taken from Rose's (65) table. They were washed, dried, weighed and cooked in a small quantity of water until tender. Adjustments in the amount of supplement were made, as with the basal ration, following each weekly weighing.

Each pup was fed separately, the day's portion of food being divided and fed twice daily while the pups were small and once daily toward the end of the feeding period. Tap water was kept in the kennels at all times. Viosterol was given once weekly except to those pups receiving high levels of cod liver oil. The dry diet was mixed with warm water and fed in the form of a mash. In most cases it was eaten promptly but in instances when a pup refused a portion of the daily allotment for several days, the amount of food given was reduced until the entire amount was eaten. Pups receiving carrots as the supplemental feeding were given the basal ration, less agar, in order not to increase the fiber content of their diet above that of the other dogs.

At the end of the experimental period, the dogs were killed by injection of chloroform into the heart, and the vitamin A content of the liver was determined in Lovibond blue units essentially according to the method of Davies (7). The fresh liver was washed free of blood, weighed on a trip balance, ground and mixed thoroughly and samples weighed for analysis. Several aliquots were taken and transferred to Erlenmeyer flasks or wide mouthed bottles containing 10 cc. of 10 per cent aqueous potassium hydroxide for each 5 grams of liver, and stored in the refrigerator until analysis could be made.

When ready for analysis, the liver samples were digested with the KOH in an electric oven at a temperature of 95 to 99°C. for seven to eight hours. At this point the mixture was somewhat turbid, due to small particles of tissue. In samples found by a preliminary extraction to be high in vitamin A content, aliquots of the total sample were used for determinations so that the range of readings in the Tintometer would fall between 4 to 6 Blue Units. It was then transferred to a separatory funnel, shaken strongly with about one-half its volume of 95 per cent ethyl alcohol, and

then with an equal volume of ether. A mixture of ethyl ether and petroleum ether was used in this extraction, at the suggestion of the S. M. A. Laboratories, to prevent the formation of troublesome emulsions. Emulsions did frequently form, however, and were broken by the addition of a small amount of alcohol. The mixture was allowed to separate and the liver tissue—potassium hydroxide layer was discarded, the ether fraction washed free of alcohol by 2 or 3 washings with distilled water and filtered by suction into a wide-necked soxhlet flask through a sintered glass funnel containing anhydrous sodium sulfate. The sodium sulfate was washed with ether and the ether extract and washings evaporated off rapidly on an electric water bath. The residue, which had the appearance of a solid fat, was dried in a suction desiccator and dissolved in specially prepared anhydrous chloroform; 0.5 cc. samples of this solution were measured into the Lovibond Tintometer cell and 2 cc. of the antimony trichloride reagent added. The resultant color was matched 20 to 30 seconds after addition of the reagent and the readings recorded. The color developed was expressed as Blue Units only. The average of three or more separate samples checking within 0.5 of one Blue Unit, was taken as the vitamin A content of the liver sample.

Experimental findings. In general, all dogs except the negative control animals were normal in appearance and action whatever the supplement given or the level of feeding. There was no apparent superiority of general health in those dogs receiving high levels of vitamin A, except that they had heavier, more lustrous coats than those animals on lower levels of vitamin A intake. The appearance of the eyes of all dogs receiving vitamin A or its precursor was normal, except in litters I and II in which some of the animals had watery eyes during the first two or three weeks. In all cases these had cleared up by the 5th week of the feeding period, and were thereafter normal in appearance.

Of the negative controls, two developed unmistakable symptoms of vitamin A deficiency. Dog A had a watery and at times purulent discharge from the eyes, throughout the feeding period of 21 weeks. This was most aggravated during the 6th to 12th weeks of the experimental period. Subsequent to the 12th week the discharge abated somewhat but the eyes remained dull and squinty. After 5 weeks of feeding this animal was noted to hold his head constantly toward the left side and to walk or run in circles when allowed out of the cage. His appetite was poor and he often refused most of his daily food allowance. He became very listless and was extremely nervous and awkward in movement. Dog V grew normally for eight weeks when several symptoms appeared rather suddenly. Both eyes began to exude a watery discharge and within a space of three days both became opaque and an ulcer formed on the left eye. He became very weak and unsteady, ran in circles and refused most of his food.

Within two weeks both eyes were badly ulcerated, and discharging pus. His coat was very rough and ragged in appearance and the hair came out in large patches. Dogs K and N showed no symptoms of vitamin A deficiency other than a slight discharge from the eyes, listlessness, and poor appetite, all of which symptoms appeared late in the feeding period.

All of the dogs except the negative controls gained in weight, and there was no apparent weight gain advantage in those animals receiving high levels of vitamin A.

Results of the determinations of vitamin A content of the livers of dogs receiving cod liver oil at levels of 20, 40, 100 and 200 U. S. P. units of vitamin A per 100 grams of body weight, or the equivalent of 20 units in the form of carotene in oil or as carrots, are shown in table 1. There was no detectable amount of vitamin A in the livers of any of the negative control animals, as evidenced by a complete absence of blue color in the reaction of the antimony trichloride reagent with the extract from 25 gram samples of liver.

When vitamin A was administered in any form at a level of 20 U. S. P. units per 100 grams of body weight, vitamin A was found present in the liver of the animals in amounts which could be determined in Lovibond Blue Units. No significant difference was found in the vitamin A content of the livers of dogs receiving 20 U. S. P. units of vitamin A in cod liver oil and in those of dogs receiving an equivalent amount of its precursor, as carotene in oil, or as carrots. Analyses of the livers of dogs fed at this level showed readings (with one exception, dog C) within the narrow range of 1.4 and 6.8 Blue Units per gram of liver. The variations noted were not always in favor of any one supplement and the storage was slightly greater in litters 1 and 2 which had the longer experimental period.

The vitamin A content of the livers of dogs receiving 40 units of vitamin A per 100 grams was higher than that of litter mates receiving 20 units. The variation was wider at this level than at the lower level of intake, ranging from 3.2–14.2 Blue Units per gram of liver. The total storage of vitamin A in animals fed at a level of 40 units was approximately 1.5 to 2 times of litter mates fed at a level of 20 units.

The vitamin A content of the livers of dogs receiving 100 and 200 U. S. P. units of vitamin A per 100 grams was in all cases much higher than that of dogs fed at the lower levels and again showed wide individual variations. Roughly, dogs fed at a level of 100 units per 100 grams had a total vitamin A content 20 times as great as litter mates fed at a level of 20 units, those receiving 200 units had a total vitamin A content 30 to 40 times that of litter mates fed at the lowest level.

Thus, it appears evident that vitamin A fed as cod liver oil or as its precursor, carotene in oil or as carrots, is practically equally well utilized by the dog. A level of 20 units of vitamin A per 100 grams of body weight,

either from animal or vegetable source, appears ample for the normal growth requirements of the young puppy, and permits a slight storage.

TABLE 1

*Vitamin A content of the livers of dogs fed equivalent amounts of vitamin A as cod liver oil, carotene in oil and carrots*

| DOG | SEX | DAILY SUP'L. PER 100 GRAMS BODY WEIGHT | AVERAGE DAILY INTAKE | INITIAL BODY WEIGHT | FINAL BODY WEIGHT | TOTAL GAIN IN WEIGHT | WEIGHT OF LIVER | TOTAL VITAMIN A CONTENT | VITAMIN A CONTENT PER GRAM OF LIVER | VITAMIN A CONTENT OF LIVER PER 100 GRAMS BODY WEIGHT |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Litter I. Feeding period 21 weeks | | | | | | |
| | | *U.S.P. units* | *U.S.P. units* | *kgm.* | *kgm.* | *kgm.* | *grams* | *L.B.U.* | *L.B.U.* | *L.B.U.* |
| A | ♂ | None | None | 1.77 | 5.89 | 4.12 | 345.0 | 0.0 | 0.0 | 0 |
| B | ♀ | 20 units C.L.O. | 886 | 1.46 | 6.18 | 4.72 | 287.0 | 1001.6 | 3.40 | 16 |
| C | ♂ | 20 units carotene | 966 | 1.43 | 7.09 | 5.66 | 380.0 | 4803.7 | 12.64 | 68 |
| E | ♀ | 20 units carrots | 822 | 1.56 | 5.67 | 4.11 | 250.0 | 1430.0 | 5.72 | 25 |
| D | ♀ | 100 units C.L.O. | 5086 | 1.65 | 7.43 | 5.78 | 278.0 | 28,537.4 | 102.66 | 384 |
| | | | | Litter II. Feeding period 22 weeks | | | | | | |
| F | ♂ | 20 units C.L.O. | 929 | 1.85 | 6.97 | 5.12 | 290.5 | 1989.3 | 6.85 | 29 |
| H | ♀ | 20 units carotene | 871 | 1.85 | 6.35 | 4.50 | 255.5 | 976.0 | 3.82 | 15 |
| G | ♂ | 20 units carrots | 1084 | 2.26 | 8.62 | 6.36 | 361.0 | 2212.2 | 6.13 | 26 |
| I | ♀ | 200 units C.L.O. | 6804 | 1.48 | 4.31 | 2.83 | * | | | |
| | | | | Litter III. Feeding period 14 weeks | | | | | | |
| K | ♀ | None | None | 1.81 | 9.64 | 7.60 | 473.0 | 0.0 | 0.0 | 0 |
| N | ♂ | None | None | 2.38 | 12.47 | 10.09 | 432.5 | 0.0 | 0.0 | 0 |
| P | ♂ | 20 units C.L.O. | 1412 | 2.55 | 12.19 | 9.64 | 657.5 | 2386.7 | 3.63 | 20 |
| Q | ♀ | 20 units carotene | 1317 | 2.10 | 11.57 | 9.47 | 623.5 | 887.1 | 1.42 | 8 |
| R | ♀ | 20 units carrot | 1405 | 2.04 | 11.45 | 9.41 | 488.0 | 722.2 | 1.48 | 6 |
| J | ♂ | 40 units C.L.O. | 3643 | 2.50 | 16.16 | 13.66 | 684.0 | 9947.0 | 14.25 | 60 |
| O | ♂ | 40 units C.L.O. | 3003 | 2.41 | 16.16 | 13.75 | 670.0 | 2260.0 | 3.38 | 14 |
| L | ♂ | 100 units C.L.O. | 9608 | 2.72 | 19.96 | 17.24 | 796.0 | 20,313.9 | 25.52 | 102 |
| S | ♀ | 100 units C.L.O. | 5811 | 1.81 | 12.42 | 10.61 | 556.5 | 19,310.5 | 34.70 | 155 |
| M | ♂ | 200 units C.L.O. | 16708 | 2.50 | 14.32 | 11.73 | 587.5 | 26,837.0 | 45.68 | 102 |
| | | | | Litter IV. Feeding period 14 weeks | | | | | | |
| V | ♂ | None | None | 1.81 | 5.89 | 4.08 | 262.0 | 0.0 | 0.0 | 0 |
| W | ♂ | 20 C.L.O. | 1139 | 1.93 | 10.43 | 8.50 | 400.0 | 1188.0 | 2.97 | 11 |
| X | ♂ | 20 carotene | 999 | 1.70 | 10.03 | 8.27 | 589.0 | 1248.7 | 2.12 | 12 |
| Y | ♀ | 20 carrot | 841 | 1.64 | 8.06 | 6.42 | 355.0 | 924.2 | 2.04 | 11 |
| U | ♂ | 40 C.L.O. | 2021 | 1.81 | 11.90 | 10.09 | 550.0 | 1771.0 | 3.22 | 15 |
| ; | ♂ | 100 C.L.O. | 4341 | 1.25 | 9.07 | 7.82 | 439.0 | 18,350.2 | 41.80 | 202 |
| T | ♀ | 200 C.L.O. | 13888 | 2.38 | 12.07 | 9.69 | 490.0 | 66,934.0 | 136.60 | 555 |
| A | ♀ | 200 C.L.O. | 7565 | 1.53 | 8.22 | 6.69 | 276.5 | 41,618.8 | 150.52 | 503 |

* Not done.

An increased intake of vitamin A above this level results in proportionately greater storage. From the data presented in table 1, it may be calcu-

lated that the young growing puppy has a minimum vitamin A requirement of roughly 15 U. S. P. units of vitamin A per kilogram of body weight. An intake of vitamin A in excess of this amount may be stored in the liver, the amount stored being directly proportional, though not arithmetically so (because of waste), to the concentration of vitamin A in the ration.

## SUMMARY AND CONCLUSIONS

Normal puppies, taken at weaning age, were fed a vitamin A deficient basal diet supplemented by vitamin A in the form of cod liver oil or in equivalent amounts of its precursor, carotene in oil, or carotene in the natural state as cooked carrots. Negative control animals from each litter developed symptoms of vitamin A deficiency.

The normal growth requirements of the young puppy were provided for, and a slight storage in the liver was permitted, by the administration of 20 U. S. P. units of vitamin A per 100 grams of body weight. At this level, cod liver oil, carotene in oil or in carrots appeared to be equally well utilized as sources of vitamin A. Greater intake of vitamin A, fed as cod liver oil resulted in a proportionately higher concentration of vitamin A in the liver of the dog. Although no weight gain advantage was afforded by the higher levels of vitamin A intake, there were evidences of better nutritive condition in the superior lustre and heaviness of coat.

## REFERENCES

(1) Grimm, P. and D. M. Short. This Journal **118:** 477, 1937.
(2) Rea, J. L. and J. C. Drummond. Ztschr. Vitamin Forsch. **1:** 177, 1932.
(3) Ralli, E., A. Pariente, G. Flaum, and A. Waterhouse. This Journal **103:** 458, 1933.
(4) Frohring, W. O. Proc. Soc. Exper. Biol. and Med. **33:** 280, 1935.
(5) Busson, A. and H. Simonnet. Compt. Rend. de Seances de la Soc. de Biol. **109:** 716, 1253, 1932.
(6) Morgan, A. F. and E. A. Garrison. J. Biol. Chem. **85:** 687, 1930.
(7) Davies, A. W. Biochem. J. **27:** 1770, 1933.

# RESPONSES OF NORMAL AND HYPOPHYSECTOMIZED IMMATURE RATS TO MENOPAUSE URINE INJECTIONS[1]

H. H. TYNDALE, LOUIS LEVIN[2] AND P. E. SMITH

*From the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City*

Received for publication June 25, 1938

Since the discovery of gonadotropic principles in the anterior hypophysis (1, 2) and in pregnancy urine (3, 4) the normal immature female rat and mouse have been almost exclusively employed in assays of gonad stimulating substances from all sources. Both quantitative (ovarian weight) and qualitative (histologic) changes have been widely used in determining the potency and the nature of such gonadotropic factors, i.e., whether follicle stimulating, luteinizing or both.

In the immature animals used for such testing, the gonadotropic secretion of the anterior pituitary gland probably is at a low level as compared with the post-puberal condition. Whether the secretory output is constant or subject to cyclic variations as in the adult is unknown. The possibility that such secretion might interfere materially with results obtained upon injection of gonadotropic substances has received relatively little attention. That the animal's own pituitary gland might be the cause of a variable error in assay work has been suggested repeatedly by Smith (5, 6) and others, on the basis of responses obtained in the hypophysectomized rat (7).

The present report is primarily a comparison of the responses of the normal and the hypophysectomized rat to injections of menopause urine extracts. Various dose levels of this substance were injected into a large series of normal and hypophysectomized immature rats and the ovarian responses of the two types of animals compared. A large group of normal rats had also been injected with pregnancy urine extract and compared with the CU[3] treated animals. The results reveal certain definite limitations to the conclusions which may be justifiably drawn from assays made on normal, immature rats.

[1] Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council.

[2] E. R. Squibb and Sons Fellow.

[3] CU is an abbreviation for human female castrate or menopause urine.

MATERIALS AND METHODS. A total of 371 rats (Long-Evans strain) was used. Of these, 164 normals were injected with menopause urine extract, 130 normals with pregnancy urine extract, and 77 were hypophysectomized and injected with menopause urine extracts. Besides these, a series of 65 normal rats from another strain were also injected with CU in order to compare the uniformity of response between strains.

Litters of 4 to 6 female rats were hypophysectomized at 25 to 41 days of age. The large majority (57 of 77) were between 27 and 34 days old at operation. At autopsy (10 days after hypophysectomy) these rats ranged in weight from 41 to 96 grams. The large majority (55 of the 77) weighed between 47 and 67 grams. Before operation, the site of the vaginal orifice was always carefully examined to insure that first estrus, with possible formation of corpora lutea, had not occurred. Corpora already present at the time of operation persist indefinitely after hypophysectomy (8), and would have obscured the results.

An interval of 5 days after operation was allowed to elapse before beginning treatment, to give adequate time for utilization or excretion of any circulating pituitary hormones.

To check the completeness of the ablation, all pituitary capsules were serially sectioned, stained with Masson trichrome, and examined microscopically. Any animal with an hypophyseal fragment, however small, was excluded. As a further check daily body weights were taken and the thyroids and adrenals were carefully dissected and weighed at autopsy.

Treatment of the normal rats was begun when they were 20 days of age. Body weights at autopsy (5 days later) ranged from 28 to 68 grams. They, as well as the hypophysectomized rats, were injected subcutaneously, once daily for 5 days, and killed 24 hours after the last injection. The 5-day instead of the usual 3-day treatment was used in order to allow ample time for luteinization to set in. This procedure necessitates a slightly higher dosage as evidenced by a series of 28 normal rats, not included in this paper, in which CU extract at several of the lower dose levels (0.6 to 3.6 mgm.) was given in 3 daily injections and the rats killed 96 hours after the first injection. In this group the ovarian weights were approximately 30 per cent to 50 per cent greater, with the same total dose, than in the 5-day treatments.

A single preparation (L411) made by the method of Levin and Tyndale (9) from pooled menopause urine was used for all animals except those of the Wistar strain and the 28 Long-Evans rats injected for comparison with them. The preparation given these latter rats (L456) was made at about the same time by the identical procedure. Both preparations had the same amount of activity per milligram of dry powder. The extracts were repeatedly assayed on immature mice, using the method of Levin and

Tyndale (10). All doses are cited as the total milligram given during the entire treatment. The pregnancy urine extract (PU) used was Follutein.[4]

Autopsies were done under a medium power (×20) binocular dissecting microscope, with light filtered through a blue ammoniacal copper sulphate solution. This made possible rather fine distinctions as to degree of vascularization and hyperemia of the follicles. Such gross inspection of ovaries, although not an infallible method of detecting luteinization, is highly reliable.[5] The main error is in ascertaining the earliest stages of peripheral luteinization. Due to this difficulty, serial sections of the ovaries of nearly all the hypophysectomized rats and of over a third of the CU injected normal animals were made. Upon examination of this material, it was found that the presence of fully developed corpora lutea had not been detected with the binocular in the case of 4 hypophysectomized animals at the very highest doses of CU (30 and 60 mgm.). On the other hand, in every case in which vascularization and hyperemia (not hemorrhage) were seen grossly in discrete follicles, luteinization was found upon microscopic examination. Thus, except for the very earliest border line cases, the above mentioned animals on the highest doses of CU presented the only examples of serious discrepancy between the gross and microscopic detection of lutein changes. The designations of luteinization in figures 1 and 2 are based upon the combined methods. Sections were made whenever the gross examination was doubtful.

The ovaries and uteri at autopsy were rapidly weighed to 0.1 mgm., the fluid in the latter having been expressed by a rolling motion of the finger from the tips of the uterine horns toward the cut cervical end with the organ on a piece of dry filter paper. The vaginae were examined before treatment was begun and again just before autopsy. Smears were also frequently made.

*Degrees of luteinization.* As has been pointed by several investigators, extensive follicle stimulation is the characteristic response in most of the CU treated animals. This precedes any luteinization. Even in cases with many corpora lutea, non-luteinized follicles usually dominate the picture.

If neither ovary of an animal showed any lutein changes in either the theca or granulosa, it has been classified as a "pure follicular" type (figs. 1, 2).[6] If a slight luteinization occurred in the theca but without the

[4] The Follutein was obtained from E. R. Squibb & Sons through the courtesy of Dr. E. A. Anderson.

[5] The large, white, opaque corpora lutea so frequently seen in ovaries of animals subjected to pregnancy urine treatment are but rarely found in the CU injected rats.

[6] A slight hypertrophy of the interstitial cells usually appears to be the very first evidence of luteinizing activity, but only under very heavy stimulation do these cells come to resemble true lutein cells and hence the early change in the interstitial tissue is disregarded in the classification used.

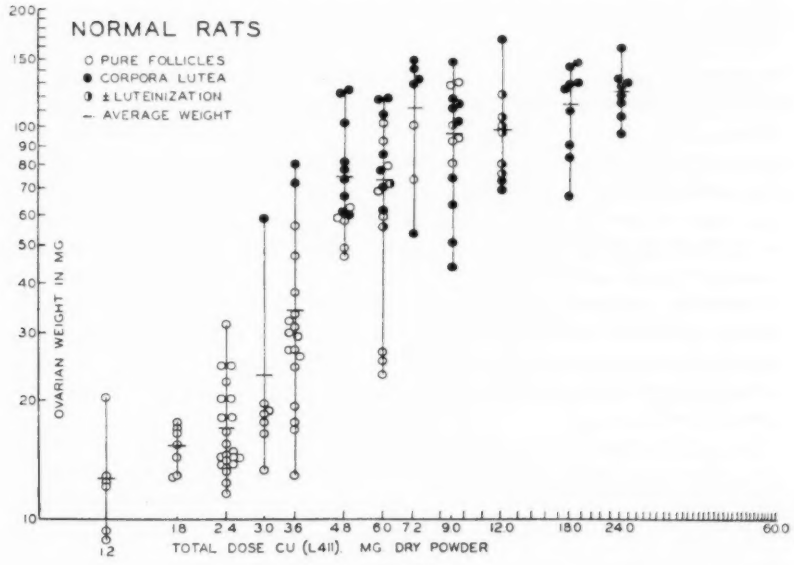

NORMAL RATS
O PURE FOLLICLES
● CORPORA LUTEA
◑ ± LUTEINIZATION
— AVERAGE WEIGHT
OVARIAN WEIGHT IN MG
200
150
100
90
80
70
60
50
40
30
20
10
12
18
24
30
36
48
60
72
90
120
180
240
600
TOTAL DOSE CU (L4II). MG DRY POWDER

Fig. 1

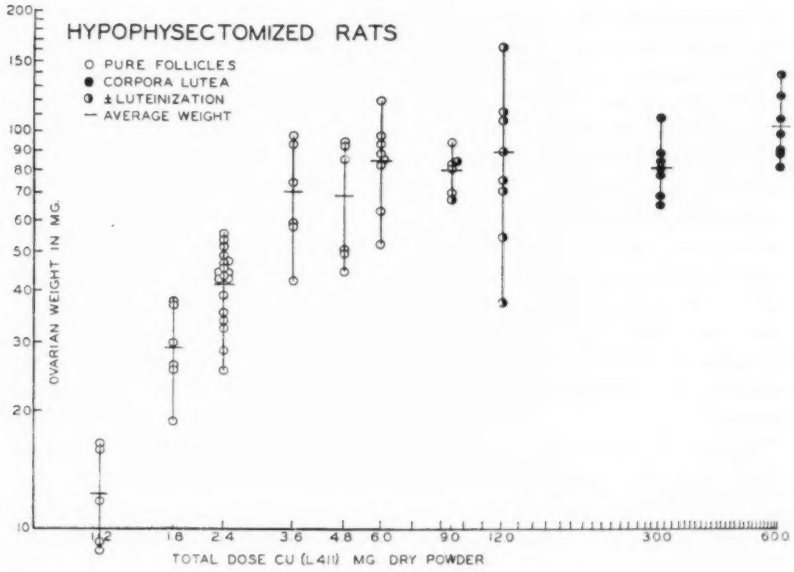

HYPOPHYSECTOMIZED RATS
O PURE FOLLICLES
● CORPORA LUTEA
◑ ± LUTEINIZATION
— AVERAGE WEIGHT
OVARIAN WEIGHT IN MG.
200
150
100
90
80
70
60
50
40
30
20
10
12
18
24
36
48
60
90
120
300
600
TOTAL DOSE CU (L4II) MG DRY POWDER

Fig. 2

formation of typical corpora the animal was included in the group of "± luteinization" even though there may have been some very small atypical corpora. If large solid corpora were present, even though the ovary still contained predominantly only large follicles, it has been considered that the intermediate (±) stage is past, and that true luteinization has set in, and the animal has been placed in the "corpus luteum" group. In such ovaries it is usually found that small lutein cysts are present and that most of the large follicles show areas of thecal luteinization.

Under the conditions of the present experiments, the above described changes appear to be essentially similar in both the normal and the hypophysectomized rats so that the classification applies to both types. The dosage which will induce luteinization differs markedly in the two experimental types, however, as does the equally important factor of the uniformity with which changes occur at different dose levels, as will be described in this paper.

In comparing the ovarian reaction of normal and hypophysectomized rats, with increasing dose levels of CU, the most outstanding difference is to be found in the variability with which luteinization occurs in the normals and the constancy of its occurrence when a certain dose level is reached in the hypophysectomized rats. As shown in figure 1 the ovaries of an occasional normal animal show typical corpora lutea with a 3 mgm. dose; the proportion of animals showing this response is increased with a dose of 4.8 mgm. but there remain ovaries of a pure follicular type with a 9 mgm. dosage. At a 12 mgm. dosage typical corpora still are not present in all the ovaries. In the hypophysectomized animals (fig. 2) no luteinization whatsoever occurs at the lower dose levels, the first evidence of luteinization being uniformly shown at the 12 mgm. level,[7] although no typical corpora are yet present. As high a dose thus is necessary to cause luteinization in the ovaries of some of the normal animals as is required in the operated ones, in others one-fourth of this dosage causes the formation of many large fully developed corpora lutea. At intermediate doses a certain percentage of the normal rats have heavily luteinized ovaries while others at the same dose show only "pure follicles." In the hypophysectomized rats, on the other hand, although there is no luteinization until the higher doses are reached, the change, when it does occur, is closely correlated to dosage increase.

Marked differences in the response to CU occur in normal rats from a single litter even though of uniform body weight.

[7] At a slightly lower dose (9 mgm.) the ovaries of 3 of the 6 animals showed slight traces of lutein change. This was confined to but a few granulosa cells. It should be mentioned that this group of animals was hypophysectomized and treated some months after the others shown in figure 2. During the intervening period the diet of the whole colony was changed, possibly explaining the fact that at the later date certain litters became sexually mature (vaginal opening and estrus) at an earlier age than had previously been noted.

*Ovarian weight and its relation to luteinization.* At the lower dose levels of CU extract the average ovarian weights of hypophysectomized rats are approximately twice those of normal rats as has been previously shown (11).[8] At intermediate dose levels the formation of corpora lutea in the ovaries of the normal animals causes an increase in their average ovarian weight for, as shown by figure 1, the luteinized normal ovary is usually, though not invariably, heavier than the follicular ovary. Thus, at these intermediate dose levels the ovaries of the two types of animals have about the same average weights. This is also true for the higher dosages, for with these higher dosages the ovaries of the normal rats are not significantly further increased. In the hypophysectomized rats the onset of luteinization does not appear to be accompanied by an increase in ovarian weight. At a total dosage of 3.6–6.0 mgm. they become as heavy as with the higher (luteinizing) dosages and also are nearly as heavy as the luteinized ovaries of normal immature rats.

*Individual variation in ovarian weight.* Since the number of animals at each dose is insufficient to allow for statistical treatment with a calculation of the coefficient of variation, the data have been plotted so as to reveal the range of variation in ovarian weights at each dose level. The range is represented by a vertical line connecting the lowest with the highest weights. A small difference (e.g., 10 mgm.) is more significant in the case of a group of small ovaries (e.g., 20 mgm. average) than in the case of a group of large ovaries (e.g., 100 mgm. average). By the use of logarithmic or "ratio" graph paper the percentage difference between the smallest and the largest pair of ovaries at a given dosage is represented by the length of the line connecting them, regardless of the absolute magnitude of the ovarian weights. Thus, by comparing the length of these lines at each dose an index of relative amounts of individual variation may be obtained.

It will be seen in figure 1 that the "spread" or variation in ovarian weight is very great in the normal rats at those doses where luteinization is beginning to occur. This extreme degree of individual variation is not found in the hypophysectomized rats at these dosage levels although there is an increase at the dosage level at which luteinization occurs.

In order to check the possibility that the great individual variation shown by the normal rats from our colony (Long and Evans) was due to

[8] The production of a greater follicular development in the hypophysectomized rats has been explained in the above cited paper as being due to reduction in the activity of the thyroid gland in the operated rats, since the simultaneous injection of CU and thyroxin in hypophysectomized rats resulted in markedly less ovarian response in these animals as compared with CU-treated, hypophysectomized littermate controls.

some peculiarity of our strain, 65 Wistar rats were injected with menopause urine (Prep. L456) at doses of 1.2, 2.4, and 4.8 mgm.[9]

The Wistar rats were divided into two groups, one of which was treated in the same manner as our normal and hypophysectomized animals (i.e., one injection daily for 5 days with autopsy 24 hours after the last injection). The other group was injected twice daily for 3 days and killed 36 hours after the last injection. Thus, the first group was under treatment over a period of 120 hours, while the second was killed 96 hours after the first injection. Both groups received the same total doses, 1.2, 2.4, and 4.8 mgm. of CU (L456). For a comparison using this same preparation, 25 rats from our colony (Long and Evans) were divided into two similarly treated groups, at 4.8 mgm. each.

In all these groups of animals, at the dosage causing luteinization in only some of the rats, the formation of corpora was accompanied by increase in ovarian weight and by great individual variation, which was almost entirely comparable in degree to that occurring in the Long and Evans strain.

The ovarian responses suggest a greater maturity in the Wistar strain. Luteinization occurred in a small percentage of cases at a dose (2.4 mgm.) which does not cause it in our rats, and the average ovarian weights were higher in the Wistar rats than in our rats. At 4.8 mgm., although the percentage of luteinization was greater in the Wistar strain, the ovarian weights were also higher even when there was no discernible lutein change.

*Relation of ovarian response to body weight.* It has been noted that the Wistar rats were larger and more mature at the weaning age than our colony rats and that their ovaries showed a greater readiness to form corpora lutea with CU treatments. It would be expected, therefore, that the ovaries of the larger animals among the Long and Evans strain would be the ones most likely to luteinize at a transition dose of CU. However, our data show no correlation between body weight and luteinization nor between body weight and ovarian weight response in normal rats, the age being constant. The influence of age has not been examined.

The data from this paper combined with those from a previous paper suggest that in hypophysectomized rats there exists an inverse relationship between body weight and ovarian weight response to CU injections. Two series of hypophysectomized rats were identically treated with 2.4 mgm.

[9] These rats were obtained through the courtesy of Dr. E. Brandt of the N. Y. State Psychiatric Institute. They had been fed on a diet containing a high percentage of yeast (5 per cent dry weight) and averaged 48 grams at weaning at 20 days of age. According to the observations of Doctor Brandt, animals in his colony come into first estrus between the ages of 32 and 36 days. This is considerably earlier than the average of the Long and Evans rats. Furthermore, it must be especially emphasized that in the latter strain the appearance of first estrus is far more variable, occurring at any time between 33 and 103 days with an average at 53 days (12).

of CU (L411). Body weights were taken at autopsy 10 days after hypophysectomy. One series of 10 animals, hypophysectomized between 27 and 35 days, had a range of body weight of 51 to 70 grams, average 57.8 grams. The other series of 12 animals, hypophysectomized at 18 days of age, ranged in body weight from 26 to 36 grams, average 30 grams. The ovaries of the larger animals averaged 39.8 mgm., while those of the smaller rats averaged 52.0 mgm.

*Comparison of CU and PU treatment in normal rats.* In normal rats PU injection produces luteinization in much smaller ovaries than does CU. With increasing dosages of PU, slight follicular enlargement is first seen but there is an early appearance of luteinization. With dosages which double ovarian weight, luteinization is present in nearly all the animals. With CU treatments, a dosage which only doubles the weight of an ovary never causes luteinization, and corpus luteum formation is usually associated with an ovarian weight increase of 5 to 8 times. The ovarian weight responses at low and intermediate dosages show less variation with PU than with CU treatment.

Discussion. If these data on the action of menopause urine on the ovaries of hypophysectomized rats are contrasted with previous reports on the effects of pregnancy urine in the same type animal (13), (14), there can be no question but that in this test animal, two qualitatively different responses are obtained with the two urinary gonadotropic substances, as has been stated earlier (7). No dosage of PU will stimulate the development of ovarian follicles, whereas CU characteristically induces the development of large and uniform-sized follicles. Quantitative factors alone do not account for the difference between CU and PU and the conclusion of Lipschütz (15) that CU is a highly diluted urine of pregnancy is unjustified. Either PU contains no follicle stimulating substance such as is present in CU or, if it is present, there must be another substance which prevents its action. CU, on the other hand, contains a large proportion of gonadotropic factor which causes follicle development and maturation. Luteinization in the hypophysectomized rats with high doses of menopause urine appears to be best explained by assuming the presence of some luteinizing substance in the CU.

The marked difference between the responses of female hypophysectomized and normal rats to CU and PU treatments seems clearly to be due to the participation, either directly or indirectly, of the hypophysis of the intact animal. That this hypophyseal factor is not a constant one is revealed by the great range in responses as regards the follicles and their luteinization which is exhibited at any moderate dose level. This variability apparently cannot be eliminated by the careful selection of animals of certain weights and is not dependent on the strain of rats used. That it is due to a pre-ovulatory hypophyseal-ovarian cycle seems not unlikely

but is difficult to determine. The much more uniform response of hypophysectomized as compared to normal rats can be ascribed to the more uniform condition of the ovaries and to the elimination of the hypophyseal factor which augments or changes the action of the castrate urine or the pregnancy urine gonadotropic principles.

The great variability in the ovarian weight responses exhibited by the normal immature female rats to injections of urinary follicle stimulating extracts (CU) greatly impairs their usefulness for assays of this type of material. Hypophysectomized rats are more useful because of their more uniform response, but both the technical difficulties involved and the variability of response, although the latter is much less than exhibited by normal animals, make then in our opinion a less desirable form for quantitative assays of CU than is the uterine test earlier described (10).

In qualitative tests directed toward the determination of whether a human urinary gonadotropic principle is similar to castrate or to pregnancy urine, the normal immature female rat undoubtedly is useful. The relative proportion of follicle growth to luteinization induced by these two types of principles differs very decidedly. With CU luteinization never occurs in ovaries whose weight has not been at least doubled and usually does not occur until the weight has been increased 5 or more times. With PU luteinization almost always takes place before ovarian weights have been doubled and large (40–100 mgm.) ovaries of a purely follicular type never are found. These differences are far more pronounced in hypophysectomized rats, for in them PU causes no follicular growth and CU induces only follicular growth until very high dosages are used. In both normal and hypophysectomized rats much larger ovaries (with or without corpora) can be obtained with menopause than with pregnancy urine preparations.

The validity of quantitative assays of the relative amounts of follicle stimulating and luteinizing principles present in a urinary extract, made on normal immature rats, can be seriously questioned. Such determinations have been repeatedly reported (16, 17, 18, 19, 20), particularly on amenorrheic, ovariectomized and post-menopausal patients. As described in this paper and as shown graphically in figure 1, there is a changing proportion between corpora lutea and pure follicles with changing dosages when the same extract is used throughout. With the preparation used, corpora lutea are formed at a 3 mgm. dosage, pure follicles are found at a 9 mgm. dosage, and some ovaries have follicles showing only a moderate degree of thecal luteinization, even at a 12 mgm. dosage. The ovarian weight change between the 4.8 mgm. and the 24 mgm. dose is very slight and would not permit a determination of the amount of extract given. In this range of dosage there is a change in the ratio of the pure follicular type to the corpus luteum type of ovary of approximately 5:10 at the 4.8 mgm. dosage to 0:10 at the 24 mgm. dosage. This changed ratio depends, it must

be emphasized, purely on dosage and is unaccompanied by a sufficient increase in weight to reveal this change in dosage. At 3.0 and 3.6 mgm. doses, an occasional corpus luteum type of ovary appears and at half the 4.8 mgm. dosage all the ovaries are of the follicular type.

Unpublished data which will form the basis for another report indicate that in the hypophysectomized rat certain mixtures of CU and PU result in augmentation of ovarian weight without luteinization. Thus, the feasibility of using the hypophysectomized rat for quantitative assays of the relative amounts of follicle stimulating and luteinizing principles in an extract also appears doubtful.

Factors such as dosage, augmentation and the length of time before autopsy would seem to preclude the making of valid assays of the relative amounts of follicle stimulating and luteinizing principles in a urinary extract. Determinations as to whether a human urinary gonadotropic principle is similar to that of pregnancy or to that of the menopause appear entirely feasible.

## SUMMARY AND CONCLUSIONS

1. Hypophysectomized rats, as compared with normal rats, show a marked difference in ovarian response to menopause urine extract injections when given the same doses of the same preparation for the same period of time. There is a wide dose range in which corpora lutea appear in some but not all of the ovaries of normal rats. Within this dosage range corpora are not formed in hypophysectomized rats. With extremely high doses, luteinization takes place in the hypophysectomized rats also. To produce the same degree of luteinization, with the preparation used, doses 8 to 10 times those required in the normal animal must be given. This indicates that the pituitary gland plays an important rôle in the luteinization of the ovary in the normal, CU treated, immature rat.

2. In the hypophysectomized rats, the occurrence and degree of luteinization is consistent with the dosage of CU. In the normal rats corpora lutea appear, at intermediate dose levels, in a certain percentage of the rats whereas the ovaries of other animals show marked follicular enlargement with no lutein changes. When corpora are present the ovaries attain a much greater average weight. There is an enormous degree of individual variation in the ovarian weight response. These findings emphasize the difficulties of using the ovaries of normal immature rats for assay of follicle stimulating substances.

3. The difference between pregnancy urine and menopause urine which is brought out sharply by the use of the hypophysectomized animal is somewhat obscured in the normal rat through the function of the test animal's own pituitary gland. In normal rats the ratio of pure follicular to corpus luteum containing ovaries varies with increasing dosage of the

same CU extract but this variation is not accompanied by corresponding weight changes. Quantitative assays of the relative amounts of follicle stimulating and of luteinizing hormone in such extracts are therefore of questionable value. The validity of such assays with hypophysectomized rats also appears to be questionable.

REFERENCES

(1) Smith, P. E. Proc. Soc. Exper. Biol. and Med. **24:** 131, 1926.
(2) Zondek, B. Ztschr. f. Geburtsch. u. Gynäk. **90:** 372, 1926.
(3) Aschheim, S. and B. Zondek. Klin. Wchnschr. **7:** 1404, 1928.
(4) Aschheim, S. Klin. Wchnschr. **7:** 1453, 1928.
(5) Smith, P. E. J. A. M. A. **104:** 553, 1935.
(6) Smith, P. E. and E. T. Engle. J. Ped. **5:** 163, 1934.
(7) Leonard, S. L. and P. E. Smith. This Journal **108:** 22, 1934.
(8) Smith, P. E. Am. J. Anat. **45:** 205, 1930.
(9) Levin, L. and H. H. Tyndale. Proc. Soc. Exper. Biol. and Med. **34:** 516, 1936.
(10) Levin, L. and H. H. Tyndale. Endocrinol. **21:** 619, 1937.
(11) Tyndale, H. H. and L. Levin. This Journal **120:** 486, 1937.
(12) Engle, E. T., R. C. Crafts and C. E. Zeithaml. Proc. Soc. Exper. Biol. and Med. **37:** 427, 1937.
(13) Selye, H., J. B. Collip and D. L. Thompson. Endocrinol. **17:** 494, 1933.
(14) Leonard, S. L. and P. E. Smith. Anat. Rec. **58:** 175, 1934.
(15) Lipschütz, A. Endocrinol. **19:** 42, 1935.
(16) Salmon, U. J. and R. T. Frank. Proc. Soc. Exper. Biol. and Med. **34:** 463, 1936.
(17) Frank, R. T. and U. J. Salmon. Proc. Soc. Exper. Biol. and Med. **33:** 311, 1935.
(18) Frank, R. T. and U. J. Salmon. Proc. Soc. Exper. Biol. and Med. **34:** 804, 1936.
(19) Hamburger, C. Acta Path. et Microbiol. Scandinav. Suppl. **17:** 1, 1933.
(20) Hamburger, C., F. Bang and J. Nielson. Acta Path. et Microbiol. Scandinav. **13:** 75, 1936.

# THE EFFECTS OF NaCl, KCl, $CaCl_2$, AND OSMOTIC PRESSURE ON THE FROG-HEART RATE

C. R. SPEALMAN

*From the Department of Physiology and Pharmacology, Medical College of Virginia, Richmond*

Received for publication June 21, 1938

While it is generally agreed that variation of the concentration of NaCl, KCl, or $CaCl_2$ in Ringer's solutions produces changes in the rate of beat of the frog or turtle heart, there are conflicting reports concerning the direction of these changes. (See Andrus and Carter, 1922; Daly and Clark, 1921, for recent studies.) Perhaps the fact that too few concentrations of a given ion have been used in these investigations is in part responsible for this lack of agreement. Also most investigators have not studied the effects over any great period of time.

In the following experiments it has been attempted to remove these two possible sources of confusion.

METHOD. The sinus venosus of the frog heart (Rana pipiens) was cannulated *in situ* with a straight glass cannula; the heart was perfused until free of blood and removed. After clamping the cannula to a ring-stand and attaching the apex of the ventricle to a heart lever, perfusion was continued by means of a siphon connecting a reservoir of Ringer's solution to the cannula. The perfusing pressure was maintained at approximately 4 cm. of water by properly adjusting the height of the reservoir.

The Ringer's solutions of various compositions were made up fresh each day and saturated with oxygen. The solution that will be referred to as normal Ringer's solution for the data of tables 1, 2, and 3, had the following composition: NaCl, 0.112, $NaHCO_3$, 0.0175, KCl, 0.0016, $CaCl_2$, 0.0015 mole per liter. The pH was between 7.8 and 8.0. For figures 1 and 2, the solution referred to as normal Ringer's was different to the extent that the NaCl concentration was 0.133 mole per liter.

The heart was perfused with normal Ringer's solution and readings taken at the end of ten and twenty minutes. The experimental solution was then substituted and readings were taken every ten minutes until any effect on the rate was apparent. If the rate did not change in the first twenty minutes, the experiment was stopped. In all doubtful cases the experiment was continued further. (If a much shorter length of time

TABLE 1

*Summary of data for $CaCl_2$ showing the relative heart rates in Ringer's solution of different $CaCl_2$ content*

Note that the increment of $CaCl_2$ content between solutions in this table varies. The data for each of the 7 hearts used are given. The solution containing $1.5\times10^{-3}$ M/L $CaCl_2$ is normal Ringer's. 0.00 indicates the heart stopped.

| M/L × $10^3$ | 0.05 | 0.10 | 0.30 | 0.50 | 1.0 | 1.1 | 1.3 | 1.5 | 1.7 | 1.9 | 2.1 | 2.3 | 3.0 | 3.5 | 4.0 | 4.5 | 5.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Actual rate / Rate in normal Ringer's | | | | | | 0.91 | 0.98 | 1.0 | 1.00 | 1.05 | 0.98 | 0.98 | | | | | |
| | | | | 0.98 | 1.06 | | | 1.0 | | | | | 1.0 | | 0.92 | 0.81 | |
| | | 0.00 | 0.91 | 1.11 | | | | 1.0 | | | | | | | | 0.60 | |
| | | | | | | | | 1.0 | | | | | | 0.96 | | 1.05 | 0.00 |
| | | 0.98 | 0.97 | | | | | 1.0 | | | | | | 1.05 | | 1.00 | 1.03 |
| | | | | | | | 0.95 | 1.0 | | | | | | 0.98 | | 0.81 | |
| | 0.81 | 0.64 | | | | | | 1.0 | | | | | | | | | |

TABLE 2

*Summary of data for KCl*

This table is constructed in the same manner as table 1. The data for each of the 16 hearts used are given. The solution containing $1.6\times10^{-3}$ M/L KCl is normal Ringers'.

| M/L × $10^3$ | 0.40 | 0.50 | 0.60 | 0.80 | 1.20 | 1.60 | 3.00 | 3.20 | 4.00 | 4.80 | 5.00 | 5.70 | 6.00 | 8.00 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Actual rate / Rate in normal Ringer's | 0.00 | | | 0.92 | | 1.00 | | 0.84 | | 0.00 | | | | |
| | 0.78 | | 1.21 | | | 1.00 | | | 0.98 | 0.87 | | 0.00 | | |
| | 0.76 | | | | | 1.00 | | | | 0.87 | | 0.53 | | 0.87 |
| | | | | | | 1.00 | 0.97 | | 1.02 | | 0.96 | | | |
| | | 0.00 | | | 0.98 | 1.00 | | | | | | | | |
| | | 0.75 | | | 1.04 | 1.00 | | | | | | | | |
| | | 0.75 | | | 0.97 | 1.00 | | | | | | | | |
| | | 0.00 | | | 0.96 | 1.00 | | | | | | | | |
| | | 0.77 | | | 1.10 | 1.00 | | | | | | | | |
| | | | | | | 1.00 | | | 1.00 | | | | 0.83 | |
| | | | | | | 1.00 | | | 1.02 | | | | 0.54 | |
| | | | | | | 1.00 | | | 0.85 | | | | 0.79 | |
| | | | | | | 1.00 | | | 0.98 | | | | 0.78 | |
| | | | | | | 1.00 | | | 0.98 | | | | | 0.00 |
| | | | | | | 1.00 | | | | | | | | 0.79 |
| | | | | | | 1.00 | | | | | | | | 0.00 |

TABLE 3

*Summary of data for NaCl*

This table is constructed in the same manner as table 1. Since $NaHCO_3$ also contributes Na ion this is included in the figures for the concentration of NaCl. The data for each of the 10 hearts used are given. The solution containing $1.3\times10^{-1}$ M/L NaCl is normal Ringer's.

| M/L × 10 | 0.69 | 0.74 | 0.79 | 0.89 | 0.90 | 1.05 | 1.10 | 1.15 | 1.30 | 1.40 | 1.51 | 1.56 | 1.61 | 1.71 | 1.81 | 1.97 | 2.12 | 2.42 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Actual rate / Rate in normal Ringer's | | 0.81 | | | | 1.12 | | | 1.00 | | 0.61 | 0.71 | | 0.59 | | | | 0.22 |
| | | | | 0.00 | | | | | 1.00 | 0.85 | 1.00 | | 0.83 | | | | | |
| | | | | 0.57 | | | 1.12 | | 1.00 | | 0.96 | | | 0.87 | | 0.66 | | |
| | | | 0.43 | | 0.69 | | 1.12 | | 1.00 | | | | 0.90 | | | | | |
| | | | | | 0.66 | | | 1.06 | 1.00 | | 1.02 | | | | 0.91 | | 0.67 | |
| | | | | | 0.94 | | | 1.07 | 1.00 | | 0.95 | | 0.75 | | 0.55 | | | |
| | | | | 0.47 | 0.94 | | | | 1.00 | | 1.03 | | | | 0.80 | | | |
| | 0.00 | | | 0.78 | | | | | 1.00 | | | | | | | | | |
| | 0.00 | | | | | | | | 1.00 | | 1.00 | | | | | | | |
| | | | | | | | | | 1.00 | | 0.95 | | | | | | | |

before the first reading were allowed, the preliminary increase in rate which usually occurs for a few moments after changing from any one solution to another was apt to be confused with the more permanent later effects. However, solutions in regions where changes from normal rate occurred were followed over a period of time, varying from one hour to several hours, to determine if any later effects would appear.) The heart was then again perfused with normal Ringer's solution and the readings taken as before. The average of the first and last readings taken with the experimental solution was expressed as a decimal fraction of the average of the two readings taken with normal Ringer's solution before and after perfusion with the experimental solution.

Even with this careful procedure it is impossible to state exactly how much the heart rate must change in order that it can be regarded as significant. In an attempt to obtain some criterion of this, we have gone over the data on the hearts used in these experiments to find how much the heart rate changed on returning to normal Ringer's solution (after the heart had been perfused with some other solution) as compared to the previous value with normal Ringer's solution. The average change was 15 per cent. Since, in our treatment of the data, the rate in any given solution is expressed as a percentage of the average rate in the normal solution taken *before* and *after* the experiment, a considerably lower percentage change is usually significant. With a good preparation probably a 5 per cent change in rate is significant; with poorer preparations perhaps a rate change of more than 10 per cent is necessary. The behavior of the individual heart must be considered; and, since our data are unselected, values obtained on the poorer preparations are necessarily included in our tables and figures.

Results. Tables 1, 2, and 3 show the results obtained by varying the concentration of $CaCl_2$, KCl, and NaCl respectively. These results are discussed in the text.

Figure 1 shows experiments carried out on the effect of NaCl similar to those above, but with the osmotic pressure held constant (equal to 0.15 M/L NaCl) by adding glucose (circles) or sucrose (solid points). This was done to study the effect of variation in NaCl alone uncomplicated by variation in osmotic pressure. This figure shows that under these conditions the rate increases with increasing concentration of NaCl.

Figure 2 shows the experimental results of varying the osmotic pressure when the Na ion concentration is held constant (0.15 M/L). The osmotic pressure was varied (increased) by adding glucose or (decreased) by substituting sodium chromate or sodium di-thionate for sodium chloride. This figure shows that the rate decreases with increasing osmotic pressure.

Discussion. The heart rate appears to be little affected by varying the concentrations of these salts over a certain range. These approximate

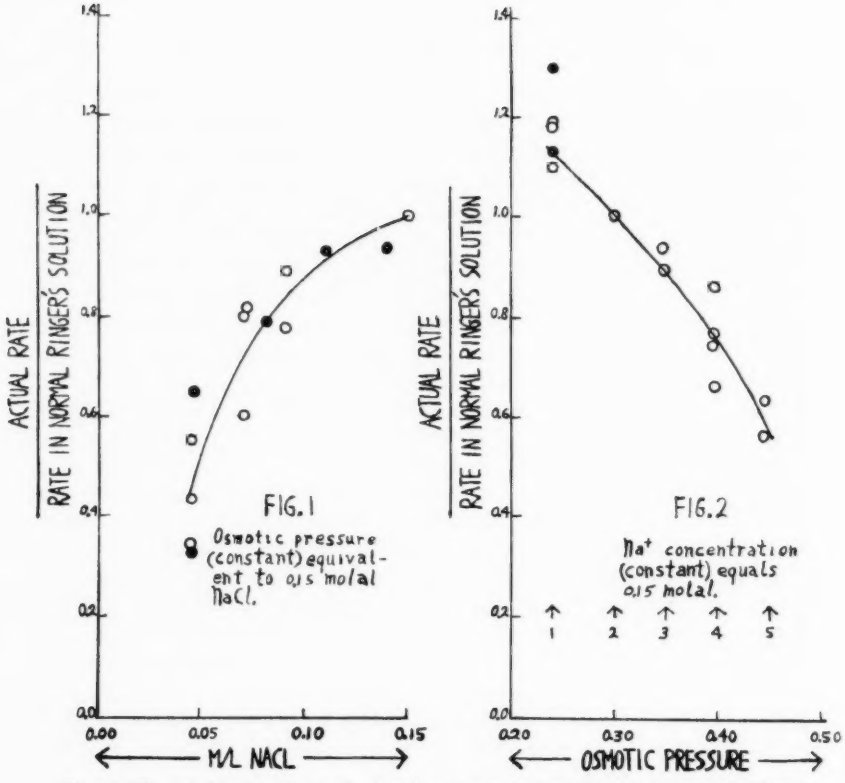



Fig. 1. The relative heart rate (actual heart rate divided by the rate obtained with normal Ringer's solution) is plotted against NaCl concentration (the $NaHCO_3$ in the Ringer's is included as NaCl). The osmotic pressure was kept constant by adding glucose (circles) or sucrose (solid points) in sufficient concentration to keep it approximately equal to that of a 0.15 M/L[1] NaCl solution. The point 0.15 on the abscissa represents normal Ringer's solution. Eleven hearts were used to obtain these data and no two points on the same abscissa were obtained with the same heart. The curve shows that the heart rate increases with the concentration of NaCl when the osmotic pressure is held constant.

Fig. 2. The relative heart rate is plotted against an osmotic pressure scale. The osmotic pressure is actually expressed as moles per liter and is equal to $\frac{P}{RT}$. The concentration of the most (osmotically) important constituents of the solutions used to obtain the various osmotic pressures (see numbered arrows above the abscissa) are: (1) 0.067 M/L $Na_2CrO_4$, circles; or 0.067 M/L $Na_2S_2O_6$, solid points, (2) 0.133 M/L NaCl, (3) 0.133 M/L NaCl, 0.05 M/L glucose, (4) 0.133 M/L NaCl, 0.10 M/L glucose, (5) 0.133 M/L NaCl, 0.15 M/L glucose. In addition the $NaHCO_3$ content (0.0175 M/L) of these solutions must be added to the above concentrations since it is of sufficient concentration to be of importance osmotically. The KCl and $CaCl_2$ content is small enough to be neglected. The position on the abscissa marked by arrow (2) represents normal Ringer's solution. Na ion concentration is equal to 0.15 M/L in all the experiments. Ten hearts were used to obtain these data and no two points on the same abscissa were obtained with the same heart. The curve shows that the heart rate changes in the opposite direction to the osmotic pressure.

ranges, expressed in moles per liter, are: $CaCl_2$, 0.0003–0.004; KCl, 0.0006–0.004; NaCl, 0.10–0.15. (See tables 1, 2, and 3.)

Outside of these ranges the heart rate, if it changes significantly, is depressed in all cases. However, this lowering is usually variable for any given concentration; and this suggests that the depressed values found outside the region of constant rate are simply due to the fact that these solutions are too abnormal to allow for proper metabolism of the heart.

In studying hearts perfused with these supposedly abnormal solutions over a period of time, it was often found that the rate became progressively slower until the heart finally stopped. Sometimes the heart would beat at a fairly constant but depressed rate. In all cases studied with these solutions the period over which the heart continued to beat was variable. Some hearts apparently would beat for an indefinitely long time, while others stopped in a very few minutes. In those cases where the heart stopped, it was found that mechanical stimulation, or better, perfusing a normal Ringer's solution through it for a few minutes, would usually cause the heart to beat again for another variable period of a few minutes to several hours when it was again perfused with the experimental solution. Sometimes the heart would begin beating apparently spontaneously. This variable behavior further indicates that this depression of rate that is obtained is probably not a specific effect of the ions concerned, but is due to the abnormality of the solutions.

Direct support of the belief that the irregularly depressed rates found in the above experiments are a result of the abnormality of these solutions was obtained in the case of NaCl in the lower concentration ranges; for, although the heart beats irregularly here, a very constant rate of beat can be gotten if the solution is made more nearly normal by maintaining the osmotic pressure with glucose or sucrose. Apparently the reason Ringer's solution containing a low concentration of NaCl does not allow the heart to function more nearly normally, is that the osmotic pressure is too low.

This finding suggested the possibility of a further study of the effect of NaCl in the lower concentration ranges. Figure 1 shows the heart rate plotted against NaCl concentration when the osmotic pressure is maintained constant (equal to that of a 0.15 M/L NaCl solution) with glucose or sucrose. This curve shows that the rate increases as the NaCl concentration increases. The values found for each solution are fairly constant for different hearts. Also the rate at any given NaCl concentration is quite constant over a period of several hours as has been determined in a number of experiments.

Since variation in NaCl concentration will cause a variation in heart rate only when the osmotic pressure is kept constant (compare fig. 1 with table 3), it must be that the osmotic pressure also influences the heart rate. In figure 2, Na ion was kept constant at 0.15 M/L. The osmotic

pressure was changed by adding different amounts of glucose to obtain osmotic pressure values greater than that of a 0.15 M/L NaCl solution. To obtain values less than that of a 0.15 NaCl solution, sodium salts of divalent anions were substituted for the NaCl of the Ringer's solution. Of the salts tried only sodium chromate ($Na_2CrO_4$) and sodium di-thionate ($Na_2S_2O_6$) met the rather exacting conditions necessary for the replacement of the NaCl in Ringer's solution. Only one osmotic pressure was used with these (see fig. 2), that obtained when the NaCl was completely replaced. The smooth curve drawn through these points show that the heart rate decreases as the osmotic pressure increases. These changes in heart rate produced by changing the osmotic pressure are not temporary; they last one or more hours. Experiments for a longer period have not been carried out.

These results indicate that NaCl concentration and osmotic pressure are important in determining the heart rate. The reason variation in the sodium chloride content without keeping the osmotic pressure constant (table 3) does not show the effect of NaCl on the heart rate is that any change in rate which should be caused by changing the NaCl concentration is almost exactly counterbalanced by the changed osmotic pressure of the solution.

All the evidence is consistent then in showing that the heart can be made to beat for a considerable time, at any of a wide range of rates, by proper variation of the osmotic pressure (Na ion constant) or of the NaCl concentration (osmotic pressure constant) of the perfusing fluid. This is in contrast to any effects obtained when KCl or $CaCl_2$ is varied.

This variation of the heart rate produced by changing NaCl concentration or the osmotic pressure of normal Ringer's solution might be assumed to be evidence that these two factors are somehow involved in the mechanism that initiates the heart beat. This assumption is one that has been generally made by investigators in this field. (See Baetjer and McDonald, 1931, for references to some of the most important papers.) While it is difficult to infer from our experiments much concerning this mechanism, it is perhaps significant that NaCl is the most abundant salt present in the heart's normal environment; for, since the first detectable change leading to the heart beat is the action current which apparently must have a physical or an electro-chemical basis, it would not be surprising that the most abundant electrolyte present would be important to such a process.

The significance of the changes in rate brought about by changing the osmotic pressure is not clear. It is possible that the changed concentration or state of some important substance of the heart tissue brought about by osmotic transfer of water accounts for the effect of the osmotic pressure on the heart rate.

SUMMARY

1. Over a certain range of sodium, potassium, or calcium chloride concentration, and without controlling the osmotic pressure, the rate of beat of the frog heart is not affected; outside this range, the heart rate is usually depressed for an uncertain period and to a variable degree. Reasons are given for supposing that this depression is not a change due specifically to any of the salts in question.

2. If the osmotic pressure is held constant, the heart rate increases rapidly with the NaCl concentration.

3. If the Na ion concentration is held constant, variation in osmotic pressure causes changes in the heart rate in the opposite direction.

4. The possible relationship of NaCl and osmotic pressure to the basic process initiating the heart beat is discussed.

REFERENCES

ANDRUS, E. C. AND E. P. CARTER. This Journal **59:** 227, 1922.
BAETJER, A. M. AND C. H. MCDONALD. This Journal **99:** 666, 1931–32.
DALY, I. DE B. AND A. J. CLARK. J. Physiol. **54:** 367, 1921.

# BLOOD POTASSIUM IN TETANY AND ASPHYXIA OF DOGS

F. J. MULLIN, JOE DENNIS AND D. BAILEY CALVIN

*From the Physiology Department of the University of Chicago and the Departments of Physiology and Biological Chemistry of the Medical Branch of the University of Texas, Galveston*

Received for publication June 29, 1938

In view of the demonstrated decrease in the potassium content of contracting muscles (Fenn and Cobb, 1936) and the increase of potassium in the blood coming from active (Kehar and Hooker, 1935) and ischemic organs (Dennis and Moore, 1938), it seemed desirable to follow the influence of anoxia and of increased muscular contraction on blood potassium in the intact animal. The recently found rôles of potassium in nerve activity and in synaptic conduction increase interest in potassium changes under these conditions. The possible influence of potassium in producing increased neuromuscular irritability under conditions of asphyxia should not be lost sight of.

METHODS. The plasma and whole blood potassium, cell volume, hemoglobin, and red cell number, as well as plasma protein nitrogen were determined in a number of dogs, first under control conditions, later after appropriate experimental treatment. Whole blood and plasma chlorides were also determined in some experiments, and the changes found to be insignificant. After anesthetization by intraperitoneal nembutal, samples of blood (15 cc. each) were drawn under oil from the exposed external jugular (care being taken to prevent contact with air) and given blind-numbers before analysis. Sodium oxalate (2 mgm. per cc. of blood) was used as the anti-coagulant. Blood potassium was determined by the method of Breh and Gaebler (1930) supplemented by the diazotization procedure of Briggs (1923). Cell volume was measured with Wintrobe tubes centrifuged for 45 minutes at 3,000 r. p. m.

One to three experiments were carried out on each dog. Neuromuscular tetany was induced by decreasing the Ca/K ratio of the cisterna magna, either by lowering the calcium ions through injection of 0.5 cc. 27 per cent sodium citrate or else by washing out the cerebrospinal fluid with a calcium-free solution or with one containing four times the normal potassium concentration, using otherwise balanced solutions and a technique previously described (Mullin, Hastings and Lees, 1938). The usual procedure

was to take a control blood sample, then to produce tetany or 2 to 4 minutes of asphyxia, and then to take another sample immediately at the height of the response. After allowing 30 to 50 minutes for recovery, the whole procedure was repeated, the "control" serving as a measure of recovery. (See protocol of typical experiment.)

In some experiments the linguo-maxillary reflex (Cardot and Laugier, 1922) was used as a test of the effect of raising the potassium level in the blood, this being produced by the injection of KCl into the carotid artery. The reflex involves the digastric muscle of the dog which contracts on appropriate stimulation of the tongue.

Results. In every case in which anoxia or tetany was produced, there was a definite increase in the potassium content of the whole blood and of the separated plasma (table 1). The rest of the experimental procedures and blood analysis was directed toward an understanding of this phenomenon.

Owing to the higher concentration of potassium in the red cells, any increased concentration of these cells would result in increased concentration of potassium in the whole blood, and since every experimental procedure used, with the exception of the administration of cyanide, was followed by an increased concentration of cells (table 1) it was necessary to determine whether this was the major factor in the blood potassium changes reported.

To this end, cell volume, hemoglobin, and red cell numbers were determined. In every instance the results were parallel. Only the red cell volumes, which were used in the calculation of concentration of potassium in the cells, are reported. Calculated values for cell potassium are included in table 1, and, after allowing for fluctuations within the limits of experimental error, reveal no significant differences between the control and experimental observations. It may therefore be regarded as established that the finding of increased potassium concentrations in whole blood and plasma results from a combination of increased concentration of red cells plus a transfer of potassium from the tissues to the plasma, and that there is no significant shift of potassium either into or out of the red cells.

Determination of plasma protein nitrogen indicated in general a slight increase in plasma proteins, consistent with the view that the increased concentration of red cells in the blood was in part the result of the loss of fluid from the blood. The findings were inconclusive, however, and since they add nothing to the analysis of the findings with respect to potassium, they are not here reported.

Tetany was regularly accompanied by an increase in the plasma potassium in a total of fourteen experiments. This increase averaged from

TABLE 1

*Effect of tetany and anoxia on blood potassium*

| | OBSERVED VALUES PLASMA K | | OBSERVED VALUES WHOLE-BLOOD K | | OBSERVED VALUES CELL VOLUME | | CALCULATED VALUES* CELL K | |
|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After |
| **Tetany** | | | | | | | | |
| | *mgm/100 cc.* | *mgm/100 cc.* | *mgm/100 cc.* | *mgm/100 cc.* | *per cent* | *per cent* | *mgm/100 cc.* | *mgm/100 cc.* |
| Cisternal high potassium | 11.7 | 13.1 | 18.1 | 23.2 | 31.9 | | | |
| | 13.3 | 16.7 | 24.3 | 25.5 | 32.2 | 35.2 | 47 | 42 |
| | 16.1 | 19.5 | 20.5 | 26.7 | 21.8 | 34.6 | 36 | 40 |
| | 15.3 | 16.3 | 20.1 | 22.3 | | | | |
| | 20.8 | 27.3 | 27.0 | 38.2 | 29.0 | 37.4 | 42 | 56 |
| Average | 15.4 | 18.6 | 22.0 | 27.2 | 28.7 | 35.7 | 42 | 46 |
| Cisternal low calcium | 13.5 | 17.2 | 21.7 | 25.3 | 41.3 | 42.0 | 33 | 36 |
| | 15.0 | 17.9 | 21.0 | 24.2 | 26.4 | 30.4 | 38 | 38 |
| | 14.8 | 15.8 | 20.6 | 21.7 | 27.9 | 28.7 | 35 | 36 |
| | 12.9 | 15.3 | 19.5 | 22.5 | 27.8 | 31.1 | 37 | 39 |
| Average | 14.0 | 16.6 | 20.7 | 23.4 | 30.8 | 33.0 | 36 | 37 |
| Cisternal sodium citrate | 15.5 | 21.1 | 21.6 | 26.2 | 32.8 | 41.4 | 34 | 33 |
| | 12.7 | 22.7 | 20.0 | 28.1 | 34.8 | 47.6 | 34 | 34 |
| | 13.7 | 19.7 | 22.8 | 30.2 | 32.2 | 38.4 | 42 | 47 |
| | 17.2 | 24.8 | 23.8 | 31.5 | 28.4 | 33.0 | 40 | 45 |
| | 19 0 | 21.4 | 27.6 | 28.8 | | | | |
| Average | 15.6 | 21.9 | 23.2 | 29.0 | 32.0 | 40.1 | 38 | 40 |
| **Anoxia** | | | | | | | | |
| Clamping trachea | 15.2 | 19.9 | 22.6 | 24.8 | 26.2 | 37.0 | 43 | 33 |
| | 16.9 | 20.5 | 22.1 | 24.1 | 31.8 | 36.7 | 33 | 30 |
| | 11.4 | 19.2 | 20.5 | 25.7 | 34.1 | 35.5 | 39 | 38 |
| | 16.1 | 17.2 | 26.9 | 28.1 | 31.5 | 35.8 | 50 | 48 |
| Average | 14.9 | 19.2 | 23.0 | 25.5 | 31.1 | 36.2 | 41 | 40 |
| Rebreathing (long tube) | 19.8 | 25.4 | 27.8 | 29.2 | 27.6 | 33.7 | 49 | 37 |
| | 11.9 | 14.0 | 22.6 | 24.6 | 46.5 | 50.5 | 35 | 35 |
| | 15.8 | 23.0 | 20.7 | | | | | |
| | 15.3 | 17.9 | 21.1 | 23.4 | | | | |
| | 14.2 | 18.1 | 19.6 | 22.6 | 30.8 | 39.4 | 32 | 30 |
| Average | 15.4 | 19.7 | 22.4 | 24.9 | 35.0 | 41.2 | 39 | 34 |

* Calculated according to following formula given by Calvin, Smith, and Mende[1] (1933):

$$D = \frac{A - (P \times C)}{B} \times 100$$

Where $A$ = Amount of constituent in 100 cc. of whole blood.
$P$ = Plasma volume, percentage in hematocrit.
$C$ = Amount of constituent in 100 cc. of plasma.
$B$ = Cell volume; percentage by hematocrit.
$D$ = Amount of constituent in 100 cc. of cells.

TABLE 1—*Concluded*

| | OBSERVED VALUES PLASMA K | | OBSERVED VALUES WHOLE-BLOOD K | | OBSERVED VALUES CELL VOLUME | | CALCULATED VALUES* CELL K | |
|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After |
| **Anoxia—*Concluded*** | | | | | | | | |
| | *mgm./100 cc.* | *mgm./100 cc.* | *mgm./100 cc.* | *mgm./100 cc.* | *per cent* | *per cent* | *mgm./100 cc.* | *mgm./100 cc.* |
| Rebreathing plus soda lime | 18.0 | 24.1 | 25.8 | 30.5 | 28.3 | 33.7 | 44 | 43 |
| | 10.5 | 12.8 | 21.0 | 23.7 | 44.7 | 45.8 | 34 | 37 |
| | 15.4 | 16.4 | 23.8 | 21.8 | | | | |
| | 15.5 | 22.0 | 22.0 | 24.7 | 30.7 | 42.0 | 37 | 28 |
| Average | 14.8 | 18.8 | 23.1 | 25.2 | 34.6 | 40.5 | 38 | 36 |
| Intravenous sodium cyanide | 14.1 | 16.8 | 24.4 | 26.1 | 40.2 | 42.2 | 40 | 39 |
| | 18.6 | 21.2 | 25.2 | 26.2 | 29.0 | 30.2 | 41 | 38 |
| | 18.4 | 19.7 | 28.8 | 30.1 | 37.5 | 34.4 | 46 | 50 |
| | 15.2 | 15.9 | 20.8 | 21.8 | 29.5 | 30.9 | 34 | 35 |
| Average | 16.6 | 18.4 | 24.8 | 26.0 | 34.0 | 34.4 | 40 | 40 |
| Total average | 15.3 | 19.1 | 22.7 | 26.1 | 32.1 | 37.1 | 39.0 | 38.8 |
| Standard deviation.. | ±2.6 | ±3.5 | ±2.7 | ±3.6 | ±5.2 | ±5.5 | ±5.0 | ±6.4 |

18 per cent on washing out calcium or increasing potassium[1] to 40 per cent on injecting citrate. The potassium change in the plasma accurately paralleled the intensity of the convulsions induced by these various procedures, citrate being always most effective. This increase in plasma potassium apparently represents an absolute augmentation from extravascular sources.

As already reported in a preliminary paper based on 22 experiments (Dennis and Mullin, 1938), asphyxia, produced by clamping the trachea, increases plasma potassium by about 30 per cent and whole blood potassium by about 15 per cent. Four additional experiments of this type

[1] Presumably the increased potassium ions in the cerebrospinal fluid do not escape into the blood under the conditions of our experimental procedure, and even so there would not be a sufficient amount to account for the rise in the blood potassium found during the tetanic state. A simple calculation will substantiate this statement. The exact concentration of potassium used in these experiments was 60 mgm. per 100 cc., and 30 cc. of solution were usually used in flushing the cisterna magna. The animals averaged about 10 kilos body weight and hence had an average calculated blood volume of 800 cc. If the entire 18 mgm. of potassium contained in the volume of solution used had escaped into the blood, it could have caused an average increase of only 2.2 mgm. per cent in the whole blood potassium of the animals used. The actual whole blood potassium increase observed in these experiments was 7.18 mgm. per cent.

(see table 1) substantiate the earlier findings.[2] Asphyxial conditions were also produced in 5 dogs by having them rebreathe through a long tube. In four of these dogs this test was combined later with the removal of carbon dioxide by soda lime. (See protocol for time relationships and typical experimental results obtained.) In these experiments the blood potassium increase was of the same magnitude (table 1) as that produced

*Typical protocol, including results.* Male dog, weight 10.1 kilogram, nembutal anesthesia.

*Procedure:* 2:05 p.m. Injected 10.1 cc. 3 per cent nembutal intraperitoneally.

2:25 p.m. Cannulated trachea and exposed external jugulars, and cisternal needle put in place.

2:30 p.m. Control sample (1) taken.

2:32 p.m. Washed out cisterna and replaced cerebrospinal fluid with a calcium-free solution. Tetany developed.

2:40 p.m. Took sample (2) at height of tetany.

2:43 p.m. Relaxed dog by replacing cisternal fluid with a balanced salt solution.

3:20 p.m. Took control sample (3).

3:30 p.m. Experimental sample (4) taken after 4 minutes' rebreathing through a long tube.

4:05 p.m. Control sample (5) obtained.

4:15 p.m. Experimental sample (6) taken after 4 minutes' rebreathing through a long tube, $CO_2$ being removed by soda lime.

*Results*

| | PLASMA K | WHOLE-BLOOD K | CELL VOLUME | HEMOGLOBIN | RED CELL COUNT | PLASMA PROTEIN-NITROGEN | CALCULATED CELL POTASSIUM |
|---|---|---|---|---|---|---|---|
| | *mgm/100 cc.* | *mgm/100 cc.* | *per cent* | | | | |
| 1. Control | 15.0 | 21.0 | 26.4 | | 4,450,000 | 1.06 | 38 |
| 2. Tetany | 17.9 | 24.2 | 30.4 | | 5,150,000 | 1.14 | 38 |
| Per cent change | (19.4) | (15.2) | (15.1) | | (15.75) | (7.5) | |
| 3. Control | 14.2 | 19.6 | 30.8 | 94.4 | 5,200,000 | 1.14 | 32 |
| 4. Anoxia | 18.1 | 22.6 | 39.4 | 121.4 | 6,500,000 | 1.14 | 30 |
| Per cent change | (27.5) | (15.3) | (27.9) | (28.7) | (25) | (0) | |
| 5. Control | 15.5 | 22.0 | 30.7 | 97.0 | 5,560,000 | 1.15 | 37 |
| 6. Anoxia | 22.0 | 24.7 | 42.0 | 117.3 | 6,830,000 | 1.14 | 28 |
| Per cent change | (42.0) | (12.3) | (36.8) | (20.9) | (22.8) | (.87) | |

by clamping the trachea, indicating that oxygen-lack rather than carbon dioxide increase is the important factor.

Sodium cyanide (10 cc. of a 0.1 per cent solution) was injected into the

[2] Confirming our results, Gerard and Tupikova (personal communication) "have found a similar rise in plasma potassium in asphyxia in dogs and frogs, accompanied, in frogs, by some loss of this ion from muscle, nerve and brain. In dogs, the tissue potassium changes were not consistent."

femoral vein in four experiments. Blood drawn at the peak of the hyperpnea showed the same potassium increase, though less marked than in direct anoxia (table 1).

As a further control, blood was drawn, in six experiments, before and after administration of nembutal. As shown in table 2, this anesthetic alone causes a fall in whole blood and plasma potassium, with a decrease in cell volume, all of these changes being in the opposite direction from those induced by the other experimental measures employed.

Over-ventilation gave negative results and showed no consistent change one way or the other (5 experiments).

Figure 1 shows the effects of injection of KCl into the carotid artery. The increase of potassium in the blood caused a very definite augmentation of the reflex activity of the animal and increased respiration markedly

TABLE 2

*Effect of nembutal anesthetization on blood potassium*

| OBSERVED VALUES PLASMA POTASSIUM | | OBSERVED VALUES WHOLE BLOOD POTASSIUM | | OBSERVED VALUES CELL VOLUMES | | CALCULATED VALUES POTASSIUM PER 100 cc. CORPUSCLES | |
|---|---|---|---|---|---|---|---|
| Before | After | Before | After | Before | After | Before | After |
| *mgm./100 cc.* | *mgm./100 cc.* | *mgm./100 cc.* | *mgm./100 cc.* | *per cent* | *per cent* | | |
| 18.5 | 16.9 | 23.4 | 22.1 | 34.1 | 32.3 | 33 | 33 |
| 15.5 | 11.4 | 23.5 | 20.5 | 37.9 | 34.1 | 37 | 38 |
| 17.0 | 13.5 | 24.5 | 21.7 | 42.4 | 41.3 | 35 | 35 |
| 20.8 | 15.1 | 37.5 | 28.0 | 38.7 | 31.5 | 63 | 56 |
| 16.3 | 11.7 | 29.1 | 24.6 | 37.5 | 36.2 | 50 | 47 |
| 20.6 | 17.2 | 26.1 | 24.3 | 34.7 | 29.0 | 37 | 42 |
| Av. 18.1 | 14.3 | 27.3 | 23.5 | 37.6 | 34.1 | 42 | 42 |

also. NaCl in the same concentration had no such effect. This heightened reflex response to a constant strength of stimulus was not always demonstrable after increasing the blood potassium in this manner, however. Injections of KCl and NaCl into the femoral vein usually gave similar results to those on arterial injection.

Discussion. The finding of increased whole blood and plasma potassium taken alone is compatible either with 1, transfer of potassium from other sources to the blood, with its distribution to the plasma alone or to the plasma and cells, or 2, increased concentration of red cells in the blood, with transfer of part of their potassium to the plasma. The calculations of table 1 indicate that there was no shift of potassium between cells and plasma, so that the increased plasma potassium found in every tetany experiment and the various types of asphyxia produced represents an actual augmentation from extra-vascular sources. Though in most cases

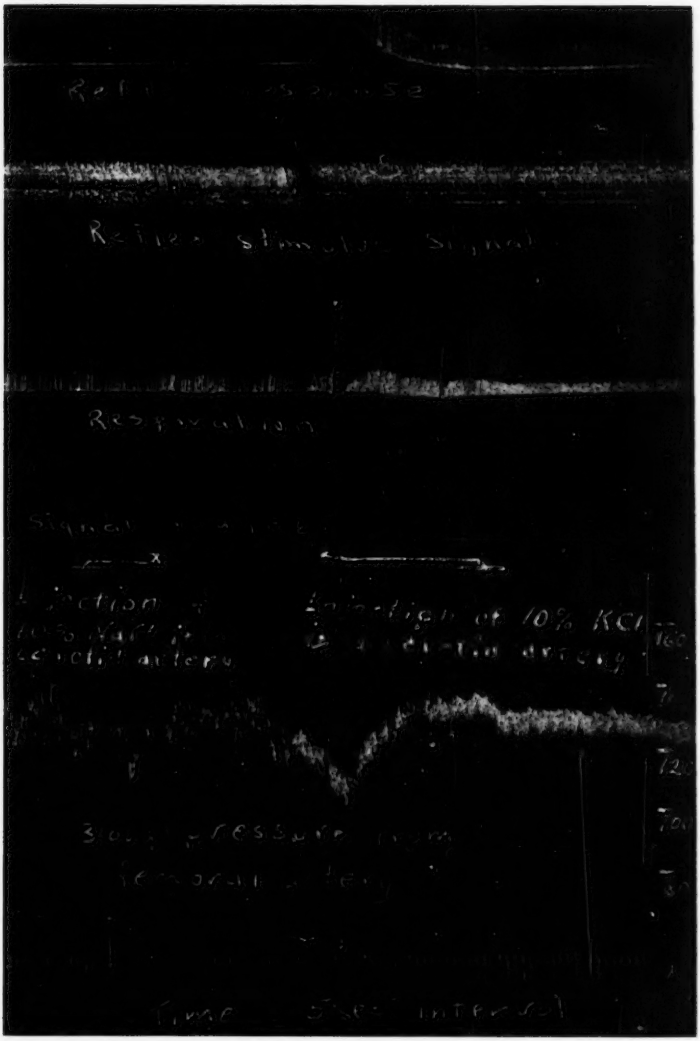


Fig. 1. The effect of injection of 2 cc. of a 10 per cent solution of KCl into the carotid artery on the magnitude of the linguomaxillary reflex. Reflex response indicates contractions of the digastric muscle on stimulating the tongue with a constant strength of induced single shock throughout the period of the record here shown. A 2 cc. injection of a 10 per cent solution of NaCl was used as a control. Nembutal was used as the anesthetic. The dog's head was tied in a fixed position on the board after the head of the muscle and tendon was isolated through a small incision for the purposes of recording its contractions by a double tambour system.

the cell potassium remained constant, there are certain exceptions to the general picture. In three of the asphyxia experiments there was a marked decrease in the calculated cell potassium. In each of these cases blood was drawn after respiratory movements had ceased and the heart had noticeably slowed and weakened. After extreme anoxia possibly erythrocyte permeability had increased and potassium leaked out into the plasma. Even in these experiments, however, the entire plasma potassium increase could not be accounted for by that escaping from the red corpuscles.

A greater relative increase in whole blood potassium was found in tetany than in asphyxia. This extra whole blood potassium of tetany (above that accountable for by the plasma increase) is due to more additional corpuscles entering the circulation, as shown by the greater percentage increase in red cell count in tetany than in asphyxia. Schafer and Moore (1896) and especially Barcroft (1925) have studied the rôle of the spleen in increasing the number of erythrocytes in the circulating blood under asphyxia and other conditions of stress. Palitz and Luckhardt (personal communication) have recently shown the ease of liberation of erythrocytes by the spleen in conditions of hyperpyrexia, and some such mechanism may also act during tetany. The changes in plasma protein nitrogen, cell volumes, and red cell numbers found in their experiments are of a similar magnitude to ours. The slightly higher calculated cell potassium of tetany may be due to this influx of erythrocytes into the circulation.

Baetjer (1935) and Fenn (1937) have shown that during activity muscles lose potassium to their environment. Houssay, Marenzi and Gerschman (1937) and D'Silva (1937) have found that potassium is mobilized from the liver under the influence of adrenalin. Either or both of these tissues could and probably do serve as a source of the increased blood potassium of the tetany or asphyxia of our experiments. In fact, Houssay and his collaborators mention an increased blood potassium after asphyxia and indicate the adrenal-liver mechanism as being involved in this response.

Asphyxial conditions increase the irritability of many reflex centers. This has been studied most recently by Porter, Blair, and Bohmfalk (1938). (See also Gerard, Arch. Neurol. and Psychiat., in press.) Our present study and previous experiments (reported by Mullin, Lees and Hastings in 1935) indicate that a relative or absolute rise in the potassium content of media bathing central neurones causes a definite increase in activity and lowering of the threshold of neuromuscular irritability. This has been shown by others also; while the experiments of Vogt (1936) and Brown and Feldberg (1936) have shown that potassium possibly plays a rôle in synaptic conduction, at least in the autonomic nervous system. Gerard and Magoun (1936) showed increased after-discharge following cerebellar stimulation in cats as a result of potassium injection. Cherbouliez and Laugier (1926) showed an augmentation of the linguo-maxillary reflex as a result of anoxia of the brain centers produced by a drop in blood

pressure. Blier and Kleitman (1930) found that no consistent augmentation of this reflex followed cephalic anemia. We have sometimes seen a slight increase in this reflex response as a result of producing asphyxia by clamping the trachea, and have definitely obtained a marked augmentation on increasing the potassium of the blood going to the head. Although this later factor may in part be explained on the basis of stimulation of peripheral nerve-elements subserving pain-sensibility (Moore, 1934), the failure of NaCl in similar hypertonic concentrations to cause a like change can be regarded as indicating that the increased potassium probably plays some more specific rôle in affecting the threshold of reflex irritability, especially since similar intravenous injections sometimes gave the same results.

The increased blood potassium found under conditions of anoxia arises from tissues and presumably from intracellular fluid. This would imply a diminished intracellular/extracellular potassium ratio and, in the case of neurones, might account for the heightened reflex activity actually seen. Even if no potassium leaves the nerve cells, an increased concentration in plasma from other sources would lead in the same fashion to increased nervous irritability. It is our belief that the potassium increases we have observed in the blood may act as a possible means of producing the lowered threshold of nervous irritability observed in these asphyxia experiments, or that these increases in blood potassium reflect still greater increases in potassium in the fluids bathing the neuromuscular apparatus. A parallel condition is seen in the rise of blood lactate following muscular exertion.

## SUMMARY

1. Increased blood potassium results from anoxemia or tetany (produced by changing the Ca/K ratio of cerebrospinal fluid) in the dog.

2. The increase cannot be accounted for on the basis of a loss of blood fluid or a potassium shift from erythrocytes to plasma.

3. It is suggested that the extra potassium entering the blood may come from active muscle or possibly the liver (adrenalin stimulation).

4. This raised plasma potassium level may serve to increase central nervous system irritability and synaptic conduction during asphyxia, and may reflect even greater localized potassium changes in the central nervous system.

5. Injection of hypertonic KCl into the carotid artery or the femoral vein sometimes leads to an augmentation of the linguo-maxillary reflex response.

## REFERENCES

BAETJER, A. M. This Journal **112:** 139, 1935.
BARCROFT, J. Lancet **208:** 319, 1925.

BLIER, Z. A. AND N. KLEITMAN. This Journal **94:** 118, 1930.
BREH, F. AND O. H. GAEBLER. J. Biol. Chem. **87:** 81, 1930.
BRIGGS, A. P. J. Biol. Chem. **57:** 351, 1923.
BROWN, G. L. AND W. FELDBERG. J. Physiol. **86:** 290, 1936.
CALVIN, D. B., A. H. SMITH AND L. B. MENDEL. This Journal **105:** 135, 1933.
CARDOT, H. AND H. LAUGIER. Compt. Rend. Soc. Biol. **86:** 529, 1922.
CHERBOULIEZ, A. AND H. LAUGIER. Compt. Rend. Soc. Biol. **94:** 1229, 1926.
D'SILVA, J. L. J. Physiol. **90:** 303, 1937.
DENNIS, J. AND R. M. MOORE. This Journal **123:** 443, 1938.
DENNIS, J. AND F. J. MULLIN. Proc. Soc. Exper. Biol. and Med. **38:** 560, 1938.
FENN, W. O. This Journal **120:** 675, 1937.
FENN, W. O. AND D. M. COBB. This Journal **115:** 345, 1936.
GERARD, R. W. AND H. W. MAGOUN. Proc. Soc. Exper. Biol. and Med. **34:** 755, 1936.
HOUSSAY, B. A., A. D. MARENZI AND R. GERSCHMAN. Compt. Rend. Soc. Biol. **124:** 383, 1937.
KEHAR, N. D. AND D. R. HOOKER. This Journal **112:** 301, 1935.
MOORE, R. M. This Journal **110:** 191, 1934.
MULLIN, F. J., A. B. HASTINGS AND W. M. LEES. This Journal **121:** 719, 1938.
MULLIN, F. J., W. M. LEES AND A. B. HASTINGS. This Journal **113:** 100, 1935.
PORTER, E. L., R. K. BLAIR AND S. W. BOHMFALK. J. Neurophysiol. **1:** 166, 1938.
SCHAFER, E. A. AND B. MOORE. J. Physiol. **20:** 1, 1896.
VOGT, M. J. Physiol. **86:** 258, 1936.

# INSULIN CONVULSIONS AFTER REMOVAL OF THE STELLATE GANGLIA[1]

R. A. PHILLIPS AND S. B. BARKER

*From the Department of Physiology, Cornell University Medical College, New York City*

Received for publication June 14, 1938

Drabkin and Ravdin (1937) state, "In thirteen experiments upon eleven animals we have obtained the unexpected result that the excision of both stellate ganglia strikingly modifies the response to large doses of insulin. These animals, though hydrated, had no convulsions after insulin. During an observation period of three to four hours they walked about the laboratory and were playful, exhibiting no sign of reaction to the drug." These experiments were all performed upon dogs. Since this observation, if it applied to cats, would influence the interpretation of studies we were pursuing, the following experiments were performed.

METHODS. Three cats and subsequently two dogs were given the same dosage of insulin administered by Drabkin and Ravdin, 20 units per kilogram body weight, intravenously. The blood sugar was followed, but we were primarily interested in ascertaining if the animals would or would not have convulsions. Care, however, was taken to standardize the experiments. The animals were fasted uniformly for 18 hours. They were not allowed to romp about the laboratory, but were placed in their cages except when blood samples were obtained (Rakestraw (1921) has shown that short periods of exercise increase the blood sugar). Blood sugars were determined by the method of Benedict (1931). All animals exhibited typical insulin convulsions and were then recovered by the administration of glucose by stomach tube.

*Cats.* Several days subsequently the right and left stellate ganglia were excised transpleurally, and two weeks later insulin was again administered in the dosage previously given. Two days later, in one cat, the superior cervical ganglia and the sympathetic chain in the neck were excised, and 3 weeks later insulin was administered as before.

*Dogs.* The right stellate ganglion was removed transpleurally, and 10 days later the left stellate ganglion was excised. Within 10 days the animals were again given insulin and observed as before.

RESULTS. A typical series of events during an experiment on a dog was as follows: Fasting blood sugar at the time of injection of insulin was

[1] Read before the American Physiological Society, April 24, 1937.

65 mgm. per cent. An hour later, the value had fallen to 38 mgm. per cent. Two hours after the injection, the sugar had reached 26 mgm. per cent, but there still were no convulsions, although the animal exhibited hyperpnea and was apathetic. A typical convulsion involving the entire body was not observed until five hours after insulin. After several minutes of frequent convulsions, glucose was administered, causing prompt recovery.

The stellate ganglia were next excised, at separate operations. A week after the second excision, both nictitating membranes were relaxed, and the fasting blood sugar was 78 mgm. per cent. One hour after insulin injection, the sugar value was 38 mgm. per cent. One hour later, the animal was restless, and clonic movements of the head, hyperpnea, and salivation indicated the early picture of hypoglycemia. Although the three-hour blood sample was down to 23 mgm. per cent, running movements and actual thrashing convulsions were not exhibited for another half-hour.

TABLE 1

*Time of occurrence of convulsions after administration of insulin (Iletin U-20*). 20 units per kilo body weight, intravenously*

| | CATS | | | | | | DOGS | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Before excision of stellate ganglia | | After excision of stellate ganglia | | After excision of cervical sympathetic chain | | Before excision of stellate ganglia | | After excision of stellate ganglia | |
| | *hours* | *minutes* | *hours* | *minutes* | *hours* | *minutes* | *hours* | *minutes* | *hours* | *minutes* |
| A | 3 | 13 | 3 | 53 | 5 | 51 | 4 | 21 | 3 | 01 |
| B | 3 | 57 | 3 | 04 | | | 4 | 55 | 3 | 39 |
| C | 4 | | 5 | 32 | | | | | | |

* Supplied by Eli Lilly and Co.

After convulsions were well established, glucose solution was injected intravenously, the animal was anesthetized with ether, and the upper thoracic and lower cervical spinal cord exposed. Stimulation by means of a Harvard inductorium (secondary coil at 6 cm.) of the right and left 1st, 2nd and 3rd thoracic roots produced no contraction of nictitating membranes nor change in pupillary size. Stimulation of each superior cervical ganglion resulted in contraction of the corresponding nictitating membrane together with dilatation of the respective pupil.

Essentially the same series of physical and chemical changes leading invariably to severe convulsions were observed in the cats following the intravenous injection of 20 units of insulin per kilo body weight. The results are summarized in table 1.

Discussion. It will be noted that convulsions were observed in all animals given insulin both before and after excision of the stellate ganglia. These results do not confirm those reported by Drabkin and Ravdin. While it is impossible to rule out the functional regeneration of pre-

ganglionic fibers to post-ganglionic neurons supplying the thyroid or pituitary, or both (these pathways were suggested by Drabkin and Ravdin for explaining their results), still the absence of nictitating membrane and pupillary response after stimulating the isolated first, second, and third thoracic roots, and the presence of these responses after stimulating the superior cervical ganglia directly, argue against the regeneration of the "suggested" pathways.

We feel that there are three possible explanations for the failure of Drabkin and Ravdin to observe convulsions. First, their dogs were observed from "three to four hours" only. As will be noted in table 1, none of our animals showed convulsions in less than 3 hours. Second, their animals "walked about the laboratory and were playful," while our animals were kept in their cages in an attempt to minimize the amount of lactic acid mobilized which could hence serve, through the synthesis of liver glycogen and its subsequent breakdown, as a source of blood sugar. Third, since no mention of feeding of the dogs is made in their experiments, the animals may have been fed shortly before or during the course of the observations.

## CONCLUSIONS

Convulsions were observed following the administration of insulin to cats and dogs both before and after the removal of the right and left stellate ganglia. These results contradict those of Drabkin and Ravdin (1937), who failed to find convulsions after excision of the ganglia. The possible errors in their experiments are discussed.

## REFERENCES

BENEDICT, S. R. J. Biol. Chem. **92:** 141, 1931.
DRABKIN, D. L. AND I. S. RAVDIN. This Journal **118:** 174, 1937.
RAKESTRAW, N. W. J. Biol. Chem. **47:** 565, 1921.

# ARSENIC IN THE NUTRITION OF THE RAT[1]

E. HOVE, C. A. ELVEHJEM AND E. B. HART

*From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison*

Received for publication June 27, 1938

Small amounts of inorganic arsenic compounds are used in medical practice as an "alterative." It is said that the arsenic has a general "tonic" effect, and is especially beneficial in the maintenance of hemoglobin and R.B.C. levels. However, positive results in the treatment of nutritional anemias with iron and arsenic salts reported before the specific nature of copper in this rôle was recognized lose much of their significance. Results from this laboratory (1) indicated that arsenic had a slight but temporary beneficial effect in anemic rats receiving adequate iron when given at a level of 1 microgram per day. The response, however, did not approach that produced by copper. A similar response to arsenic in cases of pernicious anemia was noted by Hill (2).

Although the presence of traces of arsenic in plant and animal tissue has been recognized for some time there is still a good deal of indecision as to whether it is merely an accidental and potentially dangerous contaminant (3), or a naturally occurring component of living tissues (4). If the latter view is correct, the question of its possible essential rôle in the nutrition and metabolism of animals arises.

As far as the authors are aware no positive results on growth stimulation of experimental animals when fed small amounts of arsenic have been recorded. Sollman (5) found that arsenous oxide produced a slight but progressive decrease in the growth rate of rats when fed at levels of 0.15 to 5.0 micrograms a day. This is supported by the work of Kampelman (6) who injected 0.3 to 3.0 micrograms of $As_2O_3$ into rats and noticed a histologic picture of the thyroid which indicated marked arrest of activity. Knell (7) found a fall in the basal metabolic rate of rats injected with somewhat larger amounts of the oxide. However, Coulson, Remington and Lynch (8) detected no difference in the growth rate of rats receiving a basal ration containing 0.2 mgm. of arsenic per kilo as compared with the same

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

ration to which were added 17.7 mgm. arsenic (as the arsenous oxide) per kilogram. The rates of growth and metamorphosis of tadpoles were found to be stimulated by small amounts of arsenic (9). A number of papers have reported stimulatory effects on various tissue cultures (10, 11).

Guthmann and Grass (12) give the arsenic content of the blood of normal women as 20 to 100 micrograms per 100 grams. They found considerable increases during menstruation, the first two-thirds of pregnancy and in the blood of cancer patients. They concluded that arsenic seems to have a direct relationship to growing tissue and cell proliferation.

Since mineralized cow's milk is low in arsenic we have used this as a basal ratio for the study of the relation of arsenic to growth and hemoglobin generation in the rat.

EXPERIMENTAL. The method of Hinsberg and Kiese (13) for the estimation of small amounts of arsenic was found to be very sensitive, pro-

TABLE 1

*Arsenic content of milks (Hinsberg-Kiese method)*

| BREED | As |
|---|---|
| | *micrograms/liter* |
| Guernsey: | |
| First day lactation | 71 |
| Third day lactation | 56 |
| Fourteenth day lactation | 47 |
| Holstein | 42 |
| Jersey | 49 |
| Brown Swiss | 58 |
| Goats | 55 |

ducing easily detectable stains with one-half microgram of arsenic. However the gradation in the intensity of the stain from 0.5 to 10 micrograms was not sufficiently large to allow a reading to more than 0.5 microgram. Attempts to use the ash solution directly, without first separating the arsenic as the tri-chloride generally resulted in an off-color of the stain. Recoveries of added arsenic always ran low, never being over 90 per cent and occasionally as low as 70 per cent for 5 microgram additions. The arsenic content of a series of milks, as determined by this method, is given in table 1. A more accurate but less sensitive method is the iodometric titration of Allcroft and Green (14). In this method the lowest amount of arsenic that can be accurately determined is 8 micrograms (equivalent to 0.100 cc. of the standard iodine solution). This method was used in the analysis of the blood of rats on high arsenic diets (see table 5).

As this paper was being prepared for publication the micro method for arsenic estimation of How (16) appeared. We have determined the

arsenic in cow's milk and in rat's blood, using this method (see tables 5 and 2). The milk analyzed ran somewhat lower in arsenic than milks analysed by the Hinsberg-Kiese method, but this is probably due to individual variation. The results of the analyses of whole blood and cells of rats checks well with previous results (table 5). The ashing of samples in all cases was carried out with 5 cc. $H_2SO_4$, 3 cc. perchloric acid, and 10 to 15 cc. of nitric acid.

*The relation of arsenic to the regeneration of hemoglobin.* The milk used as a basal diet in the following experiments was collected in glass containers and obtained from a single cow. The arsenic content of this milk, as determined by the Hinsberg-Kiese method, was 42 micrograms per liter. Fourteen young male rats were made severely anemic on this milk

TABLE 2

*Arsenic estimation in milk by the How method*

| SAMPLE (MILK FROM HOLSTEIN 65) | ARSENIC ADDED | ARSENIC FOUND | RECOVERY | ARSENIC/LITER |
|---|---|---|---|---|
| *cc.* | *micrograms* | *micrograms* | *per cent* | *micrograms* |
| 50 | | 1.8 | | 36 |
| 50 | | 1.9 | | 38 |
| 25 | | 1.1 | | 44 |
| 25 | | 0.8 | | 32 |
| 25 | 1 | 1.8 | 94.7 | |
| 25 | 1 | 1.9 | 99.0 | |
| 25 | 3 | 4.1 | 105.0 | |
| Blank | | 0 | | |

(hemoglobin falling to 3 grams per 100 cc. of blood in three weeks after weaning), and divided into the following seven groups:

| DAILY SUPPLEMENTS | IRON (AS $FeCl_3$) | Cu (AS $CuSO_4$) | MICROGRAMS As (AS $As_2O_3$) |
|---|---|---|---|
| | *mgm.* | *mgm.* | |
| I | 0.05 | 0.03 | 0 |
| II | 0.05 | 0.03 | 1 |
| III | 0.05 | 0.01 | 0 |
| IV | 0.05 | 0.01 | 1 |
| V | 0.05 | 0 | 0 |
| VI | 0.05 | 0 | 1 |
| VII | 0.05 | 0 | 5 |

The iron salt was prepared by dissolving reagent quality iron wire in HCl, extracting the $FeCl_3$ with ether in a continuous extractor, removing the ether and dissolving the purified $FeCl_3$ in redistilled water with 2 cc. of concentrated HCl. This solution was diluted so that 0.5 cc. contained

the indicated daily dose of iron. The arsenic supplement was prepared by dissolving resublimed $As_2O_3$ in a few cubic centimeters of concentrated HCl and diluting to one liter.

The rate of change of body weight, hemoglobin, R.B.C.'s and reticulocytes was followed for 30 days. The R.B.C.'s was determined at the end of this period. Fragility was determined by the observation of the extent of hemolysis of blood diluted 1 to 20 in NaCl solutions of 0.02 per cent increments. Since the experiment was essentially negative the data will not be presented in detail. A temporary increase in hemoglobin and R.B.C.'s was noted in the group getting iron plus 5 micrograms of arsenic daily. This increase was lost before the end of the experiment. These data are confirmatory of earlier results secured in this laboratory (1). The fragility of the R.B.C.'s was apparently not effected by these levels of

TABLE 3

*Fragility of R.B.C.'s*

The indicated daily supplements had been fed to severely anemic rats for four weeks. Note the relation between Hb and fragility.

| DAILY SUPPLEMENT | AVERAGE Hb | HEMOLYSIS IN NaCl | |
|---|---|---|---|
| | | Begins at: | Complete at: |
| | *grams per 100 cc.* | *per cent* | *per cent* |
| Fe + Cu (0.03) | 10.0 | 0.46 | 0.34 |
| Fe + Cu (0.03) + 1 microgram As | 9.7 | 0.46 | 0.34 |
| Fe + Cu (0.01) | 6.8 | 0.42 | 0.28 |
| Fe + Cu (0.01) + 1 microgram As | 8.8 | 0.40 | 0.28 |
| Fe alone | 3.3 | 0.36 | 0.24 |
| Fe + 1 microgram As | 3.4 | 0.38 | 0.22 |
| Fe + 5 micrograms As | 4.1 | 0.40 | 0.22 |

arsenic, within the limits of the method. However, an apparent relation between the fragility of the cells and the extent of the anemia is noted in table 3.

*The relation of arsenic to the growth of rats.* Young rats at weaning were placed upon a basal diet of milk mineralized with optimum levels of Fe, Cu and Mn salts. Arsenic was given in daily supplements of 0, 1 and 5 micrograms. The data on the growth rate, hemoglobin and R.B.C.'s during the six weeks period from weaning, and the fragility of the blood cells at the end of this time are given in table 4. Arsenic was without effect at the levels mentioned. The arsenic content of the whole blood cells and plasma of some of these rats is given in table 5. The blood was drawn from the aorta of the anesthetized rat with a 10 cc. hypodermic syringe, about 7 cc. being obtained from each rat.

TABLE 4

*Effect of arsenic on growth*

Hemoglobin and R.B.C. generation of rats on an all milk diet, supplemented with Fe, Cu and Mn.

| GROUP | SEX | NUMBER OF RATS | GROWTH IN 6 WEEKS' PERIOD (AVERAGE) | Hb | | R.B.C. ($\times 10^6$/mm.$^3$) | | FRAGILITY AT 6 WEEKS | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Value at 6 weeks | Change from weaning | Value at 6 weeks | Change from weaning | Begins (per cent NaCl) | Complete (per cent NaCl) |
| | | | *grams/day* | *grams/100cc.* | *grams/100 cc.* | | | | |
| Controls | ♂ | 4 | 3.04 | 13.35 | 4.40 | 926 | 467 | 0.45 | 0.35 |
| | ♀ | 3 | 2.54 | 13.74 | 4.88 | 820 | 383 | 0.47 | 0.42 |
| 1 microgram arsenic per day | ♂ | 4 | 3.07 | 13.43 | 4.59 | 966 | 494 | 0.46 | 0.40 |
| | ♀ | 3 | 2.30 | 13.45 | 4.64 | 870 | 404 | 0.48 | 0.35 |
| 5 micrograms arsenic per day | ♂ | 2 | 3.17 | 12.68 | 4.15 | 858 | 392 | 0.48 | 0.40 |
| | ♀ | 4 | 2.52 | 14.05 | 4.89 | 862 | 338 | 0.46 | 0.37 |

TABLE 5

*Arsenic content of whole blood, R.B.C.'s and plasma of rats*

| BLOOD FROM: | COMPOSITE BLOOD FROM | AVERAGE AGE | AVERAGE WEIGHT | AVERAGE Hb | HEMATOCRIT | ARSENIC CONTENT | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | R.B.C. from 100 cc. blood | Plasma of 100 cc. blood | 100 cc. whole blood |
| | *rats* | *weeks* | *grams* | *grams per 100 cc.* | *per cent* | *micrograms* | *micrograms* | *micrograms* |
| Rats on mineralized milk | 5 | 9 | 231 | 14.5 | 41.0 | 27.1 | 4.4 | 32.0 |
| | 5 | 8 | 211 | | 38.0 | 30.4* | | 35.1* |
| Rats on mineralized milk + 10 micrograms As (as Na Arsenate) | 2 | 12 | 208 | 13.8 | 45.0 | | | 145.0† |
| Rats on mineralized milk + 50 micrograms As (as Na Arsenate) | 2 | 12 | 202 | 14.0 | 47.5 | 185.0† | | 255.0† |
| Anemic rats (on milk) | 5 | 14 | 210 | 5.6 | 20.0 | 6.4 | 4.0 | 7.6 |
| | 2 | 14 | 178 | 5.5 | | | | 9.5 |
| Anemic rats (on milk) + 1 microgram As per day | 5 | 14 | 216 | 6.9 | 20.6 | 32.0 | 4.8 | 38.8 |
| | 3 | 14 | 191 | 7.2 | 21.0 | 38.6 | 15.0 | |
| | 2 | 14 | 167 | 6.5 | | | | 49.4 |
| Anemic rats (on milk) + 5 micrograms As per day | 4 | 14 | 190 | 6.6 | 19.7 | 55.1. | 42.5 | 109.0 |

* Determined by How method.

† Determined by Allcroft-Green method.

*The relation of arsenic to the rate of fall of hemoglobin.* At the conclusion of the growth studies (six weeks), the Fe, Cu and Mn salts were removed and the rats continued on milk plus the previous arsenic supplements. The changes in body weight and hemoglobin were followed for another eight weeks. The results are shown in chart 1. The arsenic content of the blood of these rats was determined (see table 5).

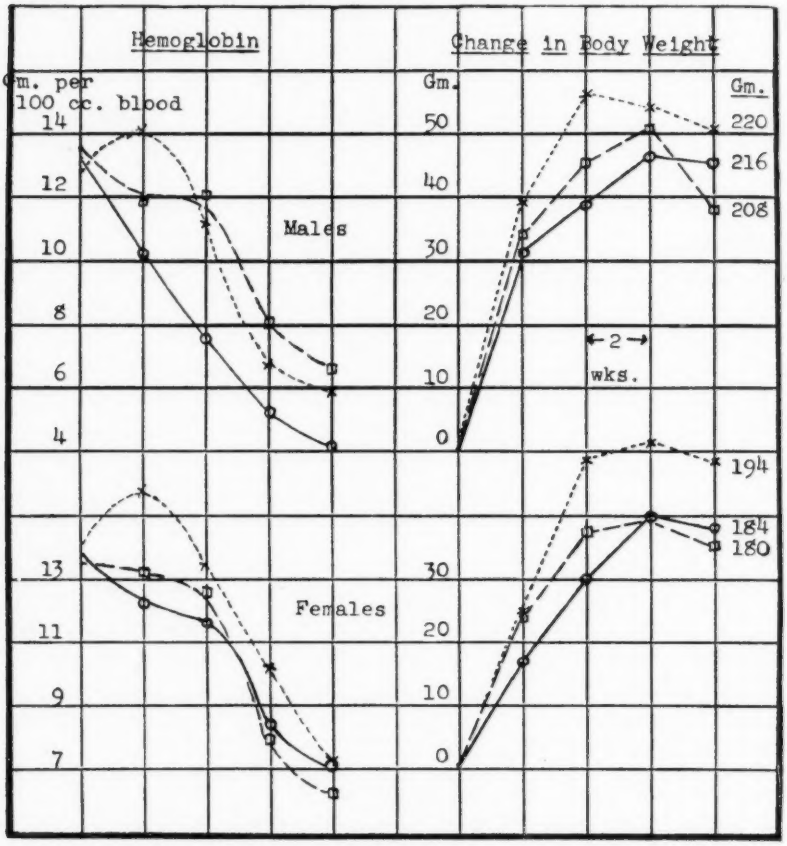



Chart 1

Solid line = Control Barred line = 1γ As/day Dotted line = 5γ As/day

*The relation of arsenic to the in vivo glycolysis by R.B.C.* Braunstein (15) has shown that in vitro glycolysis by R.B.C.'s or whole blood is greatly stimulated by sodium arsenate and to a lesser degree by sodium arsenite. The increase in inorganic phosphorus was used as one of the criteria for increased rate. We have been unable to demonstrate a similar in vivo effect by feeding 50 micrograms of arsenic (as sodium arsenate) per day to rats. The inorganic phosphorus of the blood showed no increase

over that of control rats. Analysis of the blood of these rats showed an arsenic content of 0.25 mgm. per 100 cc. The range for in vitro stimulation was found by Braunstein to be 1.2 to 37 mgm. of arsenic as sodium arsenate. The failure of the arsenic level of the blood of the rats receiving 50 micrograms daily to reach this range is sufficient to explain the negative results. This dose of arsenic is already approaching toxic proportions, so that increasing the level with the purpose of loading the blood with sufficient arsenic to demonstrate the Braunstein effect would be of little value in elucidating the physiological significance of traces of arsenic in the nutrition of the rat.

Discussion. A normal rat of 100 grams will consume about 50 cc. of milk a day. The daily intake of arsenic will be, therefore, about 2 micrograms. It is apparent from the data presented that daily intakes of 1 and 5 micrograms of arsenic in addition to that received in the milk have no effect on the growth, hemoglobin level or R.B.C. level of rats during their period of active growth. Therefore it can be concluded that if arsenic is essential for the growth and development of rats during the 6 weeks' period following weaning, 2 micrograms per day are sufficient to satisfy this requirement.

When rats are removed from the mineralized milk diet and placed on milk only, the addition of arsenic causes a slight, initial delay in the fall of the hemoglobin level; the 5 microgram arsenic supplement producing a greater delay than the 1 microgram. Since the growth of the arsenic supplemented rats was somewhat better during this period the actual differences in the rate of fall in hemoglobin are greater than the curves indicate. The mechanism of this action of arsenic may be either by increasing the life of the red blood cell through making the cell more resistant to hemolysis, or by increasing or regulating the storage of iron or copper during the growth period of the rat. Studies on the fragility of the R.B.C.'s of these rats did not indicate an increased resistance to hemolysis, although an actual effect in this direction may have been so small as to escape the sensitivity of our method. Hill (2) reports the in vitro inhibition of hemolysis by 25 mgm. of sodium arsenate per 100 cc. of blood. The occasional initial increase of hemoglobin and red blood cells that has been observed when anemic rats are placed on arsenic therapy is probably due to the same action of arsenic that causes the delay in fall of hemoglobin. There is no indication that arsenic is involved in the formation of hemoglobin, and if traces of it actually are needed to supplement the action of copper in this respect, the two micrograms daily intake through the basal milk diet fulfills this need.

The arsenic of the blood of normal rats is concentrated in the R.B.C.'s. In anemia the arsenic content of both cells and plasma drops considerably. This can readily be explained by the marked decrease in milk consumption and, hence, the arsenic intake by these rats. The addition of 1 micro-

gram of arsenic a day to the anemic rats (thus approximately restoring the normal arsenic intake) brings the arsenic partition in the blood to somewhat higher values than for normal rats. Further increase in arsenic supplements causes marked increase in the arsenic content of both cells and plasma, although the cells always retain a higher concentration than the plasma.

## SUMMARY

1. The arsenic content of cow's milk varies between 32 and 60 micrograms per liter. The concentration of arsenic in colostrum is somewhat higher than in milk.

2. At the levels of 1 and 5 micrograms per day arsenic (as $As_2O_3$ dissolved in HCl) has no effect on growth, hemoglobin levels and the red blood cell level or fragility when fed to rats on a mineralized milk diet.

3. Arsenic causes a slight initial delay in the rate of fall of hemoglobin in rats changed from a mineralized milk diet to whole milk without minerals. It has no effect on the rate of regeneration of hemoglobin or red blood cells in anemic rats when added with iron, with iron plus suboptimal copper, or with iron plus optimal copper.

4. About 80 per cent of the arsenic of the blood of normal rats is concentrated in the red blood cells. During anemia there is a marked drop in both cellular and plasma arsenic. Supplementing either anemic or normal rats with increasing levels of arsenic induces sharp increases in cellular and plasma arsenic.

5. It can be concluded that if arsenic is essential for the growth of rats during the rapid growth period or for the building of hemoglobin or red blood cells and 2 micrograms per day which is furnished by the 50 cc. of milk satisfies this requirement.

## REFERENCES

(1) Waddell, J., H. Steenbock and E. B. Hart. J. Biol. Chem. **84:** 115, 1929.
(2) Hill, L. W. Arch. Int. Med. **16:** 809, 1915.
(3) Boos, W. F. and A. B. Werby. New England J. Med. **213:** 520, 1935.
(4) Chapman, A. C. Nature **126:** 761, 1930.
(5) Sollmann, T. J. Pharmacol. and Exper. Therap. **18:** 43, 1921.
(6) Kampelmann, F. Arch. Exper. Path. u. Pharmakol. **184:** 139, 1937.
(7) Knell, E. Arch. Exper. Path. u. Pharmakol. **181:** 292, 1936.
(8) Coulson, E. J., R. E. Remington and K. M. Lynch. J. Nutrition **10:** 255, 1935.
(9) Godonnèche, J. and G. Dastugue. Bull. soc. chim. biol. **16:** 253, 1934.
(10) Sonjo, K. Folio Pharmacol. Japan **19:** 151, 1934; C. A. **29:** 3038[2], 1935.
(11) Okado, H. Folio Pharmacol. Japan **19:** 191, 271, 1934; C. A. **28:** 7276[2], 1934.
(12) Guthmann, H. and H. Grass. Arch. f. Gynakol. **152:** 127, 1933.
(13) Hinsberg, K. and M. Kiese. Biochem. Ztschr. **290:** 39, 1937.
(14) Allcroft, R. and H. H. Green. Biochem. J. **29:** 824, 1935.
(15) Braunstein, A. E. Biochem. Ztschr. **240:** 68, 1931.
(16) How, A. E. Ind. and Eng. Chem., Anal. ed. **10:** 226, 1938.

# FACTORS AFFECTING THE LOSS OF POTASSIUM FROM STIMULATED MUSCLES

WALLACE O. FENN

*From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, New York*

Received for publication July 6, 1938

In previous communications from this laboratory it has been shown that stimulation of muscles of rats, cats and frogs results in a loss of potassium in exchange for sodium and a gain of NaCl and water (Fenn et al. 1936; 1938; Fenn, 1937). This report gives the results of attempts to learn more about the significance of these electrolyte changes by determining the modifications in their magnitude which can be produced by variations of tension, mechanical movement, blood supply, and the frequency of stimulation. The results show that the potassium loss is, in general, proportional to the intensity of the contraction and is particularly favored by intermittent tetanus or an incomplete tetanus which provides rhythmical variations of tension or length.

METHODS. Cats were anesthetized with "dial," 0.65 cc. per kgm. Both sciatic nerves were exposed and cut in the sciatic notch. The large branch to the thigh muscles was cut and the peripheral end was drawn into a glass tube and through two silver wire rings for stimulating electrodes. Both Achilles tendons were freed from their insertions and attached to tension levers with strong ligature thread. The arrangement of the recording system is shown in figure 1. Both knees were fixated by strong brass pins held in a special clamp and screwed into the head of the femur from each side. The clamps were braced by steel rods to the base of the lever to prevent any movement except that of the lever. The initial tension was automatically set and maintained at a value equal to the weight, *W*, which was supported over pulleys by the tendon as shown in the diagram. When the muscle contracts, the tension developed is recorded on the lever but the weight cannot be lifted because of the pawl on the ratchet wheel, *R*, to which the cord supporting the weight is securely fastened. On the other hand as the resting muscle stretches, the weight takes up the slack by turning the ratchet wheel clockwise one notch. The resulting constancy in the initial tension has been found to be a highly important factor in obtaining reliable and uniform results for without it there is always a decrease in initial tension and therefore in height of contraction during

the course of an experiment. The lever itself consists of a strong flat steel spring, *S*, 28 cm. long, 3.8 cm. wide, and 1 mm. thick, with the muscle attached 3 cm. from the fixed end. Movements of the spring are recorded by a thread, *t*, attached to a heart lever and held taut by a light coil spring. The lever had a natural frequency of only about 10 per second but was fairly adequate for the purpose of this work since most of the contractions were tetani of about $\frac{1}{2}$ second duration. Deflections of 1 to 2 cm. were obtained for 1 kgm. of tension. A vacuum tube stimulator built by Dr. A. C. Young was used in most of the experiments and permitted use of a wide range of frequencies. The cathode was attached to the distal electrode and the central one was usually grounded.

At the end of the stimulation period the animal was bled to death by cutting the heart, and the muscles were sampled for analysis. Usually the muscles were freed from fat and nerves and a section across the middle was removed for water and potassium determinations while the remainder of the muscle was used for chloride determinations by the method of Van Slyke as modified by Manery, Hastings and Danielson (1938). For potassium determinations the samples, weighing about 1 or 2 grams, were placed in filter paper cups inside small weighing bottles. The bottle plus the dried ashless filter paper was previously weighed. The filter paper facilitated the transfer of the muscle samples to platinum crucibles for ashing after they had been dried to constant weight at 100°C. Filter paper and muscle were ashed together. The Shohl and Bennett method was used for the potassium analyses with the minor modifications previously described. All ashed samples were analyzed in duplicate for K. The average difference between these duplicate determinations was 1.5 per cent with a maximum difference of about 3 per cent. Before beginning an experiment it was customary to stimulate both sides in parallel in order to show that the response was equal on the two sides with equal stimuli and equal tension, etc. The experimental conditions of stimulation or contraction were then changed as desired and the stimulation was continued for 30 minutes or 1 hour with continuous recording of the mechanical contraction on a smoked drum.

Results. *Asphyxia.* It has been shown by Baetjer (1935) that venous blood from muscles shows an increased potassium content when the rate of blood flow is decreased to 20 per cent of its normal value. This suggests that the loss of potassium from muscles during stimulation may be the result of the relative insufficiency of the circulation for the greatly increased metabolism of exercise.

The effect of asphyxia was investigated first in resting muscles of one rat and one cat. The circulation to one leg was diminished by alternately clamping and releasing the femoral artery at intervals of a few minutes, the artery being occluded in all for 11 out of 20 minutes in the case of the

cat, and for 16 out of 30 minutes in the case of the rat. In the rat experiment the clamp was released for 10 seconds out of every minute for the last 5 minutes. Subsequent analyses of muscles from both the control and the asphyxiated sides showed no significant differences due to the asphyxia. Either the asphyxia was not sufficiently intense or the loss was too small to produce a measurable change in the potassium content of the whole muscle.

A better method of studying the asphyxia theory of potassium loss is by another type of experiment in which matched muscles of both legs were stimulated simultaneously but with the circulation through one of them artificially curtailed. Four such experiments are shown in table 1. In the first of these (no. 1) the left muscle contracted at first, less well than the right. During the stimulation the contraction in the left muscle was kept still further reduced by pressure from the finger on the artery, thinking that the resulting asphyxia would cause a loss of K greater than that found in the control. The artery was released for about 1 second out of every 10. The asphyxiated muscles did gain more $H_2O$ than the controls in spite of the smaller contraction but the loss of K was less in 3 cases out of 4.

Asphyxia presents, therefore, a striking exception to the general rule that the potassium loss and the gain in water increase and decrease together.

Similar results were obtained in the other 3 experiments which were even more convincing, for the contractions on the 2 sides were initially approximately equal in magnitude. The circulation was released for a few seconds at frequent intervals to renew the blood in the muscle for removal of K but the contraction was nevertheless kept much reduced by circulatory deficiency. In each of 11 pairs of muscles the potassium loss was greater and the water gain was less (1 exception) on the oxygenated than on the asphyxiated side. Chlorides were determined in two of the experiments but the results were contradictory. Factors affecting the intake of extracellular water with chloride probably varied in different experiments and different muscles, one of these factors being the capillary pressure on the two sides. The difficult problem of keeping the stimulation exactly equally effective on the two sides must also be considered. If however the chloride figures are used to calculate the intracellular water on the two sides there is a consistently greater intake on the asphyxiated side indicating greater metabolic increase of osmotic pressure.

The figures for the soleus muscles in table 1 are given separately because this red muscle has so frequently been found to behave differently from the other muscles of the lower leg. In this case it appears to suffer less from asphyxia and the K loss is greater on the asphyxiated side in 2 cases out of 3. Perhaps this is best correlated with the finding (see table 6)

that the lactic acid concentration during activity is much lower in the soleus than in the gastrocnemius muscles for the same amount of stimula-

TABLE 1

*Change in $H_2O$, K and Cl in equally stimulated oxygenated and asphyxiated muscles*

(Per 100 grams dry weight)

| EXPERIMENT NUMBER | MUSCLE | OXYGENATED | | | ASPHYXIATED | | |
|---|---|---|---|---|---|---|---|
| | | $H_2O$ | K | Cl | $H_2O$ | K | Cl |
| | | *cc.* | *mM* | *mM* | *cc.* | *mM* | *mM* |
| 1 | e | +28 | −8.1 | | +68 | −6.0 | |
| | t | +18 | −8.5 | | +58 | −7.6 | |
| | gm | +30 | −6.3 | | +75 | −5.0 | |
| | gl | +45 | −4.7 | | +94 | −6.8 | |
| 2 | e | +71 | −8.8 | | +114 | −4.6 | |
| | t | +71 | −9.9 | | +114 | −5.5 | |
| | gm | +73 | −6.3 | | +115 | −5.0 | |
| | gl | +86 | −6.7 | | +124 | −4.5 | |
| 3 | gm | +43 | −5.6 | +3.6 | +113 | +0.6 | +5.4 |
| | gl | +58 | −5.5 | +4.8 | +78 | +0.4 | +3.6 |
| | e | +77 | −2.6 | +3.6 | +86 | −0.1 | +4.4 |
| 4 | gm | +54 | −6.4 | +5.1 | +57 | −4.3 | +2.6 |
| | gl | +68 | −8.0 | +6.0 | +62 | −4.3 | +2.1 |
| | e | +34 | −8.9 | | +46 | −4.6 | |
| | t | +30 | −8.0 | +4.1 | +46 | −6.8 | +2.4 |
| Average..... | | +47 | −7.0 | +5.1 | +75 | −5.0 | +2.4 |
| 2 | s | +17 | −0.1 | | +58 | −4.2 | |
| 3 | s | +26 | −1.6 | | +34 | −0.4 | |
| 4 | s | +4 | −4.2 | | +6 | −4.3 | |

In each experiment 4 or 5 pairs of muscles were separately analyzed and are designated as follows: e, extensor longus digitorum; t, tibialis; gm and gl, medial and lateral gastrocnemius; s, soleus. Gains and losses were computed from average values. Five normal soleus muscles gave average values of 298 cc. $H_2O$, 40 mMols of K, while the other muscles averaged 302 cc. $H_2O$, 45 mMols of K, and 3.8 mMols of chloride. The soleus being generally different from the others was not included in the average. Dial was used in no. 4; others cats were decerebrated under ether. Weights and sex of cats in order were; 2.9 Kg ♀, 2.0 Kg ♂, 1.8 Kg ♀, and 2.4 Kg ♀. Durations of stimulations in order were 47, 36, 60,and 33 minutes. The small gains of K in no. 3 may merely indicate that in this cat the resting K content was greater than 45 m.Mols.

tion. Possibly this muscle has a relatively greater circulatory reserve so that it suffered less decrease in contraction from the experimental procedure.

On releasing the artery in experiments of this sort, the contraction recovered exceedingly rapidly so that 6 seconds were usually sufficient to triple the height of contraction and 1 or 2 seconds caused an appreciable increase. The speed of recovery was so impressive as to suggest (with Sacks and Sacks, 1935; Millikan, 1937) that oxidative energy might indeed be directly utilizable for contraction. Relaxation was delayed on the asphyxiated side.

The possibility should perhaps be considered that the diminished potassium loss in asphyxia is really due to lack of blood to carry it away. While this is a distinct possibility it nevertheless seems improbable. A small flow does persist although it is more or less intermittent and it is sufficient to permit the asphyxiated muscle to gain more water than the normally circulated one. The concentration of K in the plasma must always be far less than the concentration in the muscle fibers so that even during temporary stasis there should be no lack of diffusion gradient to carry the potassium from the fibers to the blood to the extent that it is free to move.

It seems more probable therefore that the diminished loss of K in asphyxia is related to the increased concentration of lactic acid which must be present in the muscles. While it has been supposed that the loss of K on stimulation is the result of lactic acid formation and a consequent liberation of K from combination with proteins there is better reason to suppose that the effect of lactic acid would be exactly the reverse. In the first place it is difficult to see why a decrease in the base binding power of the protein would make the potassium any more free to diffuse since the osmotic balance indicates that it must be largely ionized and exerting its independent osmotic pressure even in the resting muscle (Fenn, 1936). Secondly, a considerable amount of experimental evidence (Fenn and Cobb, 1935) indicates that the movements of potassium are determined by the opportunities which are available for exchanging with H ions so that potassium would be expected to move toward the more acid solution. According to the figures of Sacks and Sacks (1937) for cat muscles the lactic acid formed during contraction is present in the muscles in a much higher concentration than in the plasma. Even if it were equally concentrated both inside and out, it would presumably cause a greater increase in acidity in the muscle than in the blood because of the greater buffering power of the latter and we have shown in isolated frog muscles in plasma that an increase of $CO_2$ tension does make potassium move from the plasma to the muscles (Fenn and Cobb, 1935).

Although many facts appear to favor the idea that movements of K are determined by acid base requirements this theory cannot be accepted until the gain of sodium can also be explained.

*Effect of tension on potassium loss.* In a preliminary experiment to

discover whether the mechanical conditions of contraction affect the magnitude of the potassium loss both legs of a cat were similarly stimulated, one muscle contracting isometrically and the other isotonically at 1 kgm. tension. Analysis showed no difference in the potassium contents of the muscles on the two sides, the figure for the gastrocnemius muscles being 40.8 and 40.3 m. mols on the isometric and isotonic sides respectively while the figures for the soleus muscles were 36.4 and 36.5 m. mols per 100 grams dry weight. The water contents were also practically equal on both sides. It became evident, therefore, that more extreme differences in the mechanical conditions on the two sides would be necessary if a significant change in potassium loss were to be detected.

In later experiments both legs were stimulated similarly, one side being attached to an isometric lever with high initial tension while on the other side the tendon was cut and the muscle contracted under no load whatever or in some cases isotonically under a 50 gram load. Four such experiments are shown in table 2. In these experiments the soleus muscle was analyzed as a whole for K and $H_2O$. After taking a piece out of the medial and lateral gastrocnemius muscles for K and $H_2O$ the remainders of these muscles were pooled and analyzed for chloride. The tibialis and extensor longus digitorum muscles on both sides were left with their tendons attached to the ankle as controls. If the stimulation was equal on both sides these muscles should contain equal amounts of K and $H_2O$ after stimulation. The results showed that they differed by less than 2 per cent in all cases and less than 1 per cent in 3 out of 4 cases. Differences larger than this observed in the other muscles are therefore attributable to differences in the mechanical conditions of contraction. It will be seen that in 9 out of 12 pairs of muscles the high tension side apparently contained less potassium and in the other 3 cases the differences were insignificant. The average difference is 0.9 m. mol with a probable error of the mean of $\pm 0.08$. These 4 experiments were typical of the whole series. They are given in detail only because these were the only experiments in which the tibialis muscles were kept as controls. In 5 other experiments the tibialis tendon also was cut on one side and on the other side the ankle joint was either immobilized in extension or the tendon was tied to an isometric lever. In these 5 experiments, therefore, 20 pairs of muscles were analyzed in duplicate. Of these, 13 pairs showed a greater loss of potassium on the high tension side and 4 showed only an insignificantly smaller loss. The grand average of all 32 pairs of muscles in all 9 experiments gave a difference in potassium content of 1.3 $\pm 0.4$ m.mols (p.e. of mean). If the average potassium content of the unstimulated muscle is taken as 46 m.mols per 100 grams dry weight, then it may be concluded that there is a loss of 4 m.mols on the high tension side and 2.7 m.mols on the low tension side. While this difference is not far from what might

have been expected on the basis of heat production measurements in frog muscles it must be pointed out that it is only just great enough to be significant in relation to the probable error.

TABLE 2

*Effect of high and low tension on electrolyte changes*

(Per 100 grams dry weight)

| EXPERIMENT NUMBER | MUSCLE | POTASSIUM | | WATER | | CHLORIDE | |
|---|---|---|---|---|---|---|---|
| | | High T | Low T | High T | Low T | High T | Low T |
| | | Controls | | | | | |
| | | *mM* | *mM* | *cc.* | *cc.* | *mM* | *mM* |
| 6 | t and e | 34.9 | 34.2 | 380 | 386 | | |
| 7 | t and e | 41.8 | 41.5 | 404 | 398 | 11.8 | 11.8 |
| 10 | t and e | 36.8 | 36.8 | 354 | 345 | | |
| 11 | t and e | 38.8 | 39.0 | 360 | 366 | 9.0 | 9.7 |
| | | Experimental | | | | | |
| 6 | s | 36.9 | 36.0 | 370 | 373 | 11.5 | 14.8 |
| | gm | 43.4 | 43.5 | 396 | 415 | | |
| | gl | 42.7 | 42.4 | 394 | 417 | | |
| 7 | s | 37.2 | 37.9 | 342 | 329 | 9.7 | 10.9 |
| | gm | 42.5 | 45.5 | 386 | 380 | | |
| | gl | 44.0 | 46.1 | 386 | 376 | | |
| 10 | s | 38.0 | 38.5 | 329 | 327 | 7.7 | 7.9 |
| | gm | 41.6 | 43.9 | 352 | 349 | | |
| | gl | 42.9 | 44.2 | 357 | 346 | | |
| 11 | s | 40.7 | 41.1 | 343 | 337 | 11.6 | 11.1 |
| | gm | 44.4 | 44.3 | 392 | 379 | | |
| | gl | 43.4 | 45.7 | 396 | 393 | | |
| Average | | 41.5 | 42.4 | 370 | 368 | 10.1 | 11.2 |
| | | 5 other experiments—20 pairs of muscles | | | | | |
| Average | | 42.3 | 43.9 | 366 | 366 | 11.5 | 13.6 |
| Grand average | | 42.0 | 43.3 | 368 | 367 | 10.8 | 12.4 |

Muscles in 6 and 7 were stimulated for 60 and 30 min. respectively with 0.36 second tetanus (at 17 and 34 per sec. resp.) repeated every 0.9 second. Muscles in expts. 10 and 11 were stimulated for 30 minutes at 10 per second which gave an incomplete tetanus. Muscle abbreviations as in table 1.

If the average water content of resting muscles is taken as 302 cc. per 100 grams dry weight then the high and low tension muscles of table 2 gained respectively 68 and 66 cc. which is an insignificant difference. However, the figures show that in every experiment with the single exception of experiment 128, the low tension muscles gained more chloride. Assuming

that chloride is gained as an ultrafiltrate of plasma containing chloride in a concentration of 12.8 mM per 100 cc. $H_2O$ and taking 5.4 m.mols per cent dry (Fenn et al., 1938) as the initial chloride content of muscle it may be calculated from the average figures in table 2 that the extracellular $H_2O$ taken in with chloride was 42 cc. at high tension and 55 cc. at low tension, leaving 26 cc. and 11 cc. for intracellular water. Thus the gain in cellular water was probably greater where the potassium loss was greater, according to the usual rule.

The muscles represented in table 2 were stimulated in different ways as indicated in the legend. The initial tension on the high-tension side was usually 800 grams and the muscle developed another 1 to 3 kgm. of tension on stimulation. Once or twice during the half hour of stimulation both muscles were put again under the same initial tension, usually 300

TABLE 3

*Effect of duration of stimulation*

(Per 100 grams dry weight)

| TIME | EXTENSOR LONG. DIG. | | TIME | NUMBER PAIRS | GASTROCNEMIUS, ETC. | |
|---|---|---|---|---|---|---|
| | K | $H_2O$ | | | K | $H_2O$ |
| *min.* | *mM* | *cc.* | *min.* | | *mM* | *cc.* |
| 5 | −1.5 | +68 | | | | |
| 6 | −3.2 | +54 | | | | |
| 30 | −10.3 | +68 | 30* | 18 | −4.9 | +75 |
| 35 | −11.5 | +50 | 150 | 3 | −11.4 | +34 |
| 290 | −6.9 | +14 | 290 | 4 | −5.4 | +11 |

* Average of 9 cats under "dial" (previously published). Otherwise each figure is from a single decerebrate cat and represents the difference between a stimulated and an unstimulated muscle of the same animal. All given brief tetanus every 2 seconds. Results for 3 and 4 pairs were averaged for the gastrocnemius muscle but all gave very consistent results.

grams. During this time it was found in several experiments that the low tension muscle contracted much less than its mate although at the beginning it had contracted as well or nearly so. The failure of excitation, it appears, is more marked at low frequency stimulation than at high frequency. In some cases it appeared that the low tension muscle was not contracting at all, although the tibialis muscle on the same side contracted well from the same pair of electrodes. The reason for this phenomenon requires further investigation but it is evidently important for the interpretation of the results in table 2.

*Duration of stimulation.* A few figures have been obtained to show the effect of varying durations of stimulation upon the potassium loss and these are shown in table 3. The table suffers from the fact that a pair of muscles is necessary for each figure, one muscle resting and one stimu-

lated, so that a different cat was required for each duration. The loss of potassium seems to increase with increased duration at least up to 35 minutes, the apparent decrease at 290 minutes being probably due to random variations between cats. The figures show also a decreased gain of water at 290 minutes which is doubtless significant, confirming as it does some previous findings on rat muscles (Fenn and Cobb, 1936) and some other experiments on cats reported by Tipton (1938). It is striking that the gain in water is as great after 5 minutes as after 30 minutes while the loss of K is progressive, reaching a maximum at about 30 minutes.

*Intermittent and continuous tetanus.* The standard type of stimulation used in these experiments was a short tetanus every 2 seconds for half an

TABLE 4

*Effect of doubling the number of tetanic contractions per minute on water and potassium*

(Per 100 grams dry weight)

| MUSCLES | $H_2O$ | | K | |
|---|---|---|---|---|
| | 34 per min. | 68 per min. | 34 per min. | 68 per min. |
| | *cc.* | *cc.* | *mM* | *mM* |
| e | 316 | 341 | 32.6 | 32.6 |
| t | 301 | 317 | 32.6 | 33.0 |
| s | 314 | 320 | 40.2 | 37.3 |
| gm | 300 | 317 | 41.1 | 38.3 |
| gl | 330 | 353 | 39.8 | 33.6 |
| Average..... | 312 | 330 | 37.3 | 35.0 |

Three and one-tenth kgm. cat. Dec. 1936. Decerebrated under ether. Period of stimulation 35 minutes. Harvard inductorium. Circuit closed through left sciatic nerve 68 times per minute, first in series with right sciatic and then in series with a corresponding resistance so that both muscles gave about the same height of contraction (even at the end) but the right one contracted only 34 times per minute. Left muscle failed more promptly when the circulation was stopped. Serum K increased from 13.1 to 14.2 mgm. per cent as a result of the stimulation. Abbreviation of muscles as in table 1.

hour. In one experiment one leg of a cat was stimulated with a short tetanus 34 times a minute while the other leg was stimulated with a tetanus of similar duration and frequency but repeated 68 times a minute. The results (table 4) show, as expected, that there is a greater loss of potassium and a greater gain of water in the muscle stimulated the greater number of times.

From this experiment the next step was to stimulate one leg with a continuous tetanus for 30 minutes while the other leg was stimulated with an intermittent tetanus, i.e., a half-second tetanus every second or every 2 seconds. The results of these experiments were so surprising that a large number of them have been tried in order to make the result quite certain.

It was found that *a greater loss of potassium and a greater gain of water can be produced by an intermittent tetanus than by a continuous one*, although the latter lasts in all about 4 times as long. The results of one typical experiment are given in table 5. Four pairs of muscles were analyzed. With the single exception of the water contents of the soleus muscles it may be said that there was a greater gain of water and a greater loss of potassium on the side which was stimulated intermittently than on the continuously stimulated side. This finding is confirmed in the figures for the average loss of K and gain of $H_2O$ from 11 similar experiments. These average differences are large except in the case of the soleus muscle where the loss of K was nearly as great during continuous as during intermittent stimulation. This "red" muscle being adapted to more prolonged efforts and having perhaps a better circulation may be supposed to suffer *less*

TABLE 5

*The effects of intermittent and continuous tetanus on electrolyte changes*

(Per 100 grams dry weight)

| Muscle | TYPICAL EXPERIMENT NO. 5 | | | | SUMMARY OF 11 EXPERIMENTS | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Water content | | K content | | Number of pairs | $H_2O$ gain | | K loss | |
| | Intermittent | Continuous | Intermittent | Continuous | | Intermittent | Continuous | Intermittent | Continuous |
| | *cc.* | *cc.* | *mM.* | *mM.* | | *cc.* | *cc.* | *mM.* | *mM.* |
| gm | 390 | 371 | 39.9 | 44.4 | 22(5)* | +66 | +65 | −4.4 | −2.4 |
| gl | 398 | 365 | 40.2 | 45.0 | | | | | |
| t and e | 407 | 382 | 36.6 | 41.9 | 17(2) | +73 | +64 | −8.6 | −5.3 |
| s | 359 | 360 | 39.8 | 40.3 | 11(5) | +24 | +33 | −5.0 | −4.8 |
| Normal | 302 | | 45.6 | | | | | | |

* Exceptions as to K in ( ).

*diminution in K loss* when the tetanus becomes continuous than is the case with the "white" muscles. Even in the "white" muscles the result is not always consistent with the average change. Thus in the gastrocnemius muscles, as indicated in table 5, there were 5 out of 22 pairs of muscles which did not show a greater loss of K with intermittent tetanus. These exceptions are partly attributable to experimental error and partly to random differences between matched muscles or nerves resulting in inequalities in muscle response even with equal stimulation on both sides. They may also mean that the time curves for the loss of K with these two types of stimulation have different shapes, the K loss being greater with continuous stimulation for short times and greater with intermittent stimulation for long times. Thus the curves would cross and the time of crossing may vary in different cats and under varying experimental conditions. However that may be, it seems justifiable to conclude from table 5 that

cat muscles in general lose more K and gain more $H_2O$ when tetanized once or twice a second than when tetanized continuously.

It is instructive to compare these K losses with other quantities measured on the same muscles and listed in table 6. The gain in water is about equal on the two sides. The greater average chloride gain on the continuously stimulated side indicates that more of the water gained on that side is extracellular but the chloride figures were too variable for this small difference to be significant. The product of tension by the time during which that tension was maintained was always greater on the continuously

TABLE 6

*Average effects of intermittent and continuous stimulation*

| | EXPERIMENT NO. 5 ALL MUSCLES | | 11 EXPERIMENTS GASTROCNEMIUS | |
|---|---|---|---|---|
| | Intermittent | Continuous | Intermittent | Continuous |
| $\Delta H_2O$, cc | +94 | +68 | +66 | +65 |
| $\Delta$ K, mM | −6.5 | −2.7 | −4.4 | −2.4 |
| $\Delta$ Cl, mM | +4.0 | +3.3 | +2.3 | +3.7 |
| $\Delta$ cell $H_2O$ | +63 | +42 | +48 | +3.6 |
| Tension, kgm | 1.2 | 0.8 | 2.2 | 1.3 |
| Tension × time, kgm. min | 15 | 27 | 24 | 50 |
| Relative number impulses | 1.0 | 1.2 | 1 | 1–4 |
| Duration tetanus, min | 13 | 32 | — | — |
| Lactic acid, mgm. per cent | — | — | 659 | 644 |

The chloride figures represent a single determination in experiment 5 assuming 5.4 m.eq. per 100 grams dry for normal content; in the summary of 11 experiments 16 chloride figures were averaged from 7 of the experiments. The small difference in chloride between the two sides is not statistically significant. The lactic acid figures in mgm. per 100 grams wet weight are averages of 6 determinations in 2 cats. In the same 2 cats the lactic acid contents of the soleus muscles were 56 and 77 mgm. per cent for intermittent and 95 and 122 mgm. per cent for continuous stimulation. In experiment 5 each side was stimulated at a frequency that gave maximal effect, i.e., 40/sec. intermittent and 22/sec. continuous. The muscles were tetanized on the intermittent side 0.38 second every 0.95 second. $H_2O$, K and Cl calculated per 100 grams dry weight.

stimulated side although the average tension was less. The amount of tension which could be continuously maintained depends of course upon the frequency of stimulation during the tetanus. (Briscoe, 1931: Davis and Davis, 1932). In most experiments the frequency was kept optimal for both sides and it was found that a lower frequency was required on the continuous side, enough lower in fact, so that the total number of impulses was usually approximately equal on the two sides. In other experiments the frequency was kept equal on the two sides. The duration of each

individual tetanus was accurately determined in the later experiments by a rotating commutator. It may be said in general that tension was well maintained during the 30–60 minutes of stimulation at a level about one-half that initially reached and the frequency was not so high as to cause appreciable inhibition at the myoneural junction. Rough comparison with figures for heat production of muscles in developing and maintaining tension for varying periods would indicate that the total heat production must have been greater on the continuously stimulated side. Lactic acid contents[1] were determined in 2 of these experiments on 6 pairs of muscles but there was no consistent evidence for more lactic acid on the continuously stimulated side as had been anticipated. (See average figures in table 6.) It was found, however, that the lactic acid content of the soleus muscle was very much less than that of the gastrocnemius muscle, a fact which perhaps may be correlated with its unusual behavior in regard to potassium. This would suggest that muscles do not show a diminished loss of K with continuous as compared to intermittent stimulation except when the lactic acid concentration becomes elevated.

From these experiments it must be concluded that the loss of potassium is not proportional to the total energy production of the muscle, the total number of nerve impulses delivered, or to the tension time. The only quantity which seems to vary with the potassium loss is the average tension developed. This conclusion does not seem altogether satisfactory, however, and it might be pointed out that the to and fro movement of the muscle which is stimulated intermittently may be an important factor in liberating the potassium.

To check the possibility that the potassium is in some sense "shaken" out of the muscle by the rhythmical movement an experiment was so arranged that a 1 kgm. weight was alternately raised and lowered by a motor-driven eccentric, the weight being supported by one of the gastrocnemius muscles of a cat during the time of its descent. Thus the muscle was alternately stretched and released 28 times per minute for one hour. Upon analysis there was found only an insignificant excess of K and of Cl on the stretched side, neither of the muscles having been stimulated at all. It is possible however, that a similar alternating movement of the stimulated muscle would have had a different result.

The possibility might also be considered that during continuous stimulation the circulation was diminished but that the massaging action assisted the circulation during intermittent tetanus. If this were the case, however, there should have been some signs of asphyxia on the continuously stimulated side where the circulation was at least adequate to permit the maintenance of a greater tension-time and hence presumably

[1] For these analyses the muscles were frozen in situ in $CO_2$ snow and ether before sampling.

of a greater energy turnover. Although Anrep, Blalock and Samaan (1934) have found a decreased blood flow during isometric contractions of short durations it has been shown by Kramer (1936) that the blood flow is nevertheless increased in contractions of longer duration, reaching a maximum in 1.5 minutes. This was confirmed by Millikan (1938). Nevertheless, it may be well to point out that a relatively slower circulation in continuous stimulation would cause just the result observed and would also explain the anomalous behavior of the soleus muscle, which from the experiments on asphyxia, seemed to have a better circulation than the other muscles.

As additional confirmation of the greater effectiveness of intermittent tetanus as a means of liberating potassium from stimulated muscles, the figures of table 7 may be presented. The table shows the average losses

TABLE 7

*Summary of K and $H_2O$ changes due to stimulation*

(Per 100 grams dry weight)

| TETANUS | MINUTES | NUMBER PAIRS | NUMBER CATS | $H_2O$ | K |
|---|---|---|---|---|---|
| | | | | *cc.* | *mM.* |
| Continuous | 30-60 | 8 | 4† | +47 | −2.5 |
| | 33-60 | 7 | 2 | +45 | −1.8 |
| | 42 | 2 | 1* | +22 | −3.4 |
| Intermittent (every 1 or 2 sec.) | 30-60 | 7 | 2 | +55 | −4.6 |
| | 30-60 | 11 | 4* | +71 | −5.6 |
| | 30 | 18 | 9 | +75 | −4.9 |

* Decerebrate—all others under Dial.

† Previously used for salivary gland stimulation.

of potassium and gains of water in 22 cats used for stimulation experiments previous to those already reported in this paper. On collecting these figures together it appeared that the potassium loss was uniformly greater when intermittent stimulation was employed. In earlier experiments continuous stimulation had been used because it seemed logical to believe that this would prove more fatiguing to the muscle and would therefore certainly cause a greater loss of potassium. The result shows, however, that the potassium loss is greater if the muscle can be kept contracting well for a long time without excessive fatigue. This conclusion drawn from experiments on cats was also found valid for experiments on frogs (Fenn, 1937). It is also evident from table 7 that the gain of water is greater where the potassium loss is greater and that the magnitude of electrolyte changes in decerebrate animals compares favorably with that observed in anesthetized animals.

*Frequency of stimulation.* In a previous report (Fenn and Cobb, 1936) dealing with electrolyte changes in rat muscles on stimulation, data were presented showing a maximum of water gain and of potassium loss at a frequency of stimulation of 8 to 15 per second. Similar but more con-

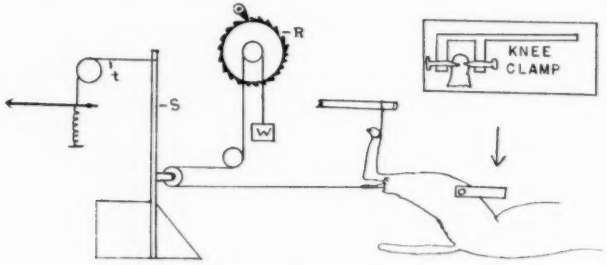



Fig. 1. Diagram of recording apparatus

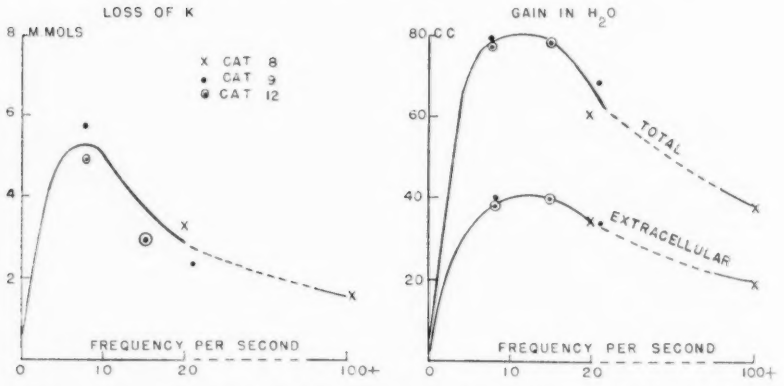



Fig. 2. Loss of K, gain of $H_2O$ and gain of chloride during stimulation for 30 minutes at various frequencies. Chloride is expressed as cubic centimeters of extracellular water assuming 12.8 m.eq. chloride per 100 grams of water. Each point represents the average from analyses on 4 muscles, medial and lateral gastrocnemius, soleus. All 3 cats weighed between 2.2 and 2.5 kgm. Cat 9 was a male, the others females. All values calculated per 100 grams dry weight of muscle. Losses and gains were computed from average normal values which were taken as 44.2 m.mols for K, 302 cc. for $H^2O$ and 5.4 m.mols for chloride.

sistent data from 3 cats are plotted in figure 2. Each point represents the average of 4 muscles, the gain or loss being estimated by subtraction from the average normal content of cat muscles.[2] Both legs of the cats

[2] All four muscles showed the same qualitative difference as to K in each of the 3 experiments. The muscles sampled were medial and lateral gastrocnemius, soleus,

were stimulated for equal lengths of time and at equal initial tensions but at different frequencies. The maximum loss of K or gain of Cl and $H_2O$ occurs at a frequency of about 8 per second where the muscles gave discrete twitches or an incomplete tetanus. At 15 or 20 per second the contractions were fused to a complete tetanus. At the highest frequency of 100 to 130 per second there was some inhibition at the myoneural junction so that an increase in the height of contraction could always be obtained by lowering the frequency. In the case of frogs a similar decrease in potassium loss at high frequencies has been observed (Fenn, 1937). It was a surprise, however, to find in two experiments a larger loss of potassium at 8 per second than at 20. It is clear, however, that this agrees perfectly with the difference found between intermittent and continuous tetanus (see table 5).

Nevertheless, the experiments of table 5 differed in one important respect from those of figure 2 for in the latter the tension was less at 8 per second than at 20 per second, the figures being 1.1 kgm. compared to 1.9 kgm. in experiment 8 and 2.2 compared to 3.0 kgm. in experiment 12. In this case therefore, the loss of K is greater where the tension, the tension $\times$ time, the number of impulses and presumably the lactic acid and total energy are all less.

Discussion. These experiments were begun with the idea that the loss of K was entirely due to metabolic changes resulting from contraction. Some observations can be explained on this basis. If the magnitude of the contraction is diminished by a diminished blood flow the loss of K also decreases. A free contraction under no load results in a decrease in the mechanical response and a small decrease in the loss of K. To a limited extent also an increase in the frequency of response (up to 8 per second) or an increase in the duration of the stimulation period results in a corresponding increase in K loss. Likewise an increase in the frequency of tetanic stimulation sufficient to cause a decrease in the magnitude of the tension maintained results in a similar decrease in the magnitude of the potassium loss and the water gain.

On the other hand, there are two observations which do not agree with the idea that the magnitude of the contraction alone determines the potassium loss. Thus there is a smaller loss of potassium with a complete tetanus at 15 or 21 per second continuously maintained for 30 minutes than with an incomplete tetanus at 8 per second maintained for a similar length of time. There is also a smaller loss with a continuous than with an intermittent tetanus. In both these cases there is a smaller loss of potassium where the magnitude of the contraction, as judged by total energy or tension $\times$ time, is greater. In the latter case the tension also is less.

and tibialis plus extensor longus digitorum. The soleus gave the same general type of curve as the other muscles.

Some subsidiary hypothesis is evidently necessary. The anomalous cases both involve an excessive loss of potassium when the muscle tension is undergoing rhythmical fluctuations. The development of tension involves a certain amount of shortening since the contraction is not strictly isometric. This excessive liberation of potassium under these conditions might be related to the excess liberation of energy in shortening and performing work (Fenn, 1924) or it might take place in a more simple mechanical manner as if the potassium were somehow "shaken" out of the fibers.

As an alternative hypothesis it might be supposed that the loss of potassium is related to the process of myoneural transmission rather than to the contraction itself. Since the intensity of these processes would in general be proportional to the magnitude of the mechanical response such an hypothesis would probably explain satisfactorily the general parallelism between the magnitude of the response and the loss of potassium and might also explain the exceptional cases of non-parallelism. But however probable it may appear that some potassium is liberated from the myoneural junction during activity along with acetylcholine (Dale, Feldberg and Vogt, 1936) as is the case apparently in the sympathetic ganglion (Vogt, 1936), it nevertheless seems difficult to believe that as much as 15 per cent and in some cases even larger fractions of the total potassium in the muscle can *all* come from the myoneural junctions. Most of this potassium must come from the interior of the muscle fibers, where it can hardly function in the processes of excitation. On the basis of present evidence, therefore, it seems most probable that the loss of potassium is in general proportional to the magnitude of the contraction and results from processes associated with contraction but that this loss is for some reason particularly favored by a fluctuating rather than a constant tension or by the small alternate shortenings and lengthenings resulting from such fluctuations in tension.

## SUMMARY

1. The loss of potassium from stimulated muscle is somewhat less when the tendon is cut than when the muscle pulls against an isometric lever at high initial tension.

2. Curtailment of the circulation through a stimulated muscle, thus partially asphyxiating it, decreases the loss of K but increases the gain of water. The loss of K is therefore not in proportion to the amount of lactic acid formed.

3. The gain of water is less after long periods of stimulation (4.5 hours) than after short periods of 30 minutes.

4. The loss of K is increased if the number of tetani per second is doubled, but it is diminished if the muscle is tetanized continuously.

5. The gain of water is about the same for 5 minutes as for 30 minutes of stimulation and is the same for continuous as for intermittent tetanus.

6. The soleus muscle contains less lactic acid for a given amount of stimulation than the gastrocnemius muscle and suffers less decrease in potassium loss from curtailment of the circulation or from continuous tetanus in place of intermittent.

7. The loss of K, gain of $H_2O$, and gain of chloride are all at a maximum at a frequency of stimulation of about 8 per second (incomplete tetanus). A complete tetanus has less effect and an inhibited tetanus at still higher frequency still less.

8. It is concluded that the loss of K is in general proportional to the magnitude of the contraction but is particularly favored by rhythmical contractions. The physico-chemical mechanism which brings about the loss of K is not yet certain.

## REFERENCES


ANREP, G. V., A. BLALOCK AND A. SAMAAN. Proc. Roy. Soc. B **114:** 223, 1934.

BAETJER, A. N. This Journal **112:** 139, 1935.

BRISCOE, G. J. Physiol. **71:** 292, 1931.

DALE, H. H. W., FELDBERG AND M. VOGT. J. Physiol. **86:** 353, 1936.

DAVIS, H. AND P. DAVIS. This Journal **101:** 339, 1932.

FENG, T. P., L. LEE, C. MENG AND S. WANG. Chinese J. Physiol. **13:** 79, 1938.

FENN, W. O. J. Physiol. **58:** 373, 1924.
Physiol. Rev. **16:** 450, 1936.
Proc. Soc. Exper. Biol. Med. **37:** 71, 1937.

FENN, W. O. AND D. M. COBB. This Journal **112:** 41, 1935.
This Journal **115:** 345, 1936.

FENN, W. O., D. M. COBB, J. F. MANERY AND W. R. BLOOR. This Journal **121:** 595, 1938.

KRAMER, K. AND W. QUENSEL. Pflüger's Arch. **239:** 620, 1936.

MANERY, J. F., I. S. DANIELSON AND A. B. HASTINGS. J. Biol. Chem. **124:** 359, 1938.

MILLIKAN, G. A. Proc. Roy. Soc. B **123:** 218, 1937.
J. Physiol. **93:** 20p., 1938.

SACKS, J. AND W. C. SACKS. This Journal **112:** 565, 1935.
This Journal **118:** 697, 1937.

TIPTON, S. R. Proc. Am. Physiol. Soc. 50th meeting, p. 203, Baltimore, 1938.

VOGT, M. J. Physiol. **86:** 258, 1936.

# THE METABOLISM OF CALCIUM AND PHOSPHORUS AS INFLUENCED BY THE ADDITION TO THE DIET OF SALTS OF METALS WHICH FORM INSOLUBLE PHOSPHATES

JAMES H. JONES

*From the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia*

Received for publication June 18, 1938

As early as 1908 Stoeltzner (1) observed that the addition of strontium salts to the diet produced a rachitic-like condition in animals. Lehnerdt (2) confirmed and extended these results and Shipley, Park, McCollum, Simmonds and Kinney (3) reported in 1922 that cod liver oil was ineffective in preventing the so-called strontium rickets. Hess (4) later stated that animals were not protected against the changes produced by strontium by "liberal amounts" of irradiated ergosterol. More recently Guyatt, Kay, and Branion (5) found that beryllium carbonate in amounts of approximately 0.5 per cent or more of the diet produced very severe rickets in rats. Cod liver oil and irradiated ergosterol were ineffective as preventive agents. These workers have concluded that beryllium acts by uniting with phosphorus in the intestinal tract to form an insoluble phosphate with the consequent loss of phosphorus to the body and the development of low-phosphorus rickets. Jones (6) subsequently found that dogs given a diet containing beryllium carbonate develop rickets which was prevented by neither cod liver oil nor ultra violet light. Deobald and Elvehjem (7) were able to produce rickets in chickens by giving either aluminum or iron salts. They agree with Guyatt et al. in respect to the manner in which these metals act. Sobel, Goldfarb and Kramer (8) on the other hand have concluded that beryllium has a direct inhibiting effect on the process of calcification.

With the possible exception of the effect of liberal iron feeding on the development of infantile rickets, as pointed out by Deobald and Elvehjem, it is doubtful if clinical rickets is ever caused by an excess of these metals. Their mode of action, however, is of experimental interest, and furthermore they serve as a useful tool in studying the metabolism of calcium and phosphorus. In the present paper are recorded a number of observations made during the past few years and dealing with various phases of this subject. As salts of different metals have been used the more inclusive term of metal rickets has been adopted to describe this condition.

EXPERIMENTAL. *A. Influence of large doses of irradiated ergosterol on metal rickets.* Since it was previously shown in this laboratory (9), by using diets containing at most only traces of calcium, that this element can be transferred from the ossified portion to the growing parts of the bone, it was thought desirable to conduct a similar experiment using a diet free of phosphorus. As the preparation of such a diet is difficult an attempt was made to substitute a diet in which the phosphorus was made unavailable by the addition of metals which form insoluble phosphates. All workers are agreed that even in the presence of liberal quantities of vitamin D little or no calcification takes place in the growing bone when diets containing any of these metals are being fed. Sobel and associates (8) refer to this condition as incurable rickets. In all of these experiments, however, comparatively small doses of vitamin D were given. The following experiments were planned to test the ability of large amounts of irradiated ergosterol to cure or prevent metal rickets and to determine if the phosphorus came from the food or was transferred from one part of the bone to another in the event ossification took place.

Preliminary experiments, in which either aluminum acetate or beryllium carbonate was used as the metal, gave definite evidence that liberal amounts of vitamin D as irradiated ergosterol could produce healing of metal rickets. The prevention of rickets under similar circumstances was then studied in greater detail. A total of 31 young rats was placed on diet 502 which is the Steenbock (10) stock diet[1] modified to contain 2 per cent yeast and to which was added 1 per cent of beryllium carbonate. Fifteen of these animals were given 5000 I. U. of vitamin D per day as irradiated ergosterol while the remaining 16 rats received no vitamin supplement. The administration of the activated ergosterol was begun 2 days after the initial feeding of the beryllium-containing diet. Nineteen of these rats were on experiment for 23 days and 12 for 16 days. At the end of each period the animals were killed by bleeding and the sera were analyzed for calcium and inorganic phosphate (Clark and Collip, 11; Gunther and Greenberg, 12). The right femur of each animal was removed for ash analysis and the wrist bones were examined by the line test technique. Besides the above 31 animals an additional 6 were given the stock diet without beryllium, 3 of which were given daily 5000 I. U. of vitamin D as above. In table 1 are given in detail the results from the 6 animals receiving the stock diet and 6 receiving beryllium. Of the latter, 3 received irradiated ergosterol and 3 did not. These 12 rats were all on experiment for 23 days and were from either one of two litters. A summary of all the results obtained from the 31 animals is also given in the form of averages in table 1. As the initial weights of most of the animals were between

[1] The casein used in these diets was kindly furnished by Smith, Kline and French, Inc., Philadelphia.

40 and 50 grams only the gains in weight are given. Although the amount of vitamin administered was far above the therapeutic dose, it was below the toxic level as growth on the stock diet was not lessened by the addition of the vitamin, but the calcium of the serum appears to have been raised slightly while the serum phosphate remained unchanged. There is also no particular difference in the degree of calcification between the 2 groups on the stock diet (with and without vitamin D) as shown by the amount and percentage of bone ash. By comparison of the animals on the stock

TABLE 1

*Effect of irradiated ergosterol on femur-ash content of beryllium-fed rats*

| RAT NUMBER | DIET | IRRADIATED ERGOSTEROL I.U.* | GAIN IN WEIGHT | SERUM | | FEMUR-ASH | |
|---|---|---|---|---|---|---|---|
| | | | | Ca | PO4-P | Weight | Per cent |
| | | | *grams* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm.* | |
| 1 | Stock | 0 | 69 | 10.7 | 8.8 | 84.3 | 50.3 |
| 2 | Stock | 0 | 78 | | | 94.4 | 51.8 |
| 3 | Stock | 0 | 88 | 11.7 | 9.7 | 92.8 | 50.1 |
| 4 | Stock | 5000 | 83 | 12.3 | 8.9 | 100.2 | 52.3 |
| 5 | Stock | 5000 | 86 | | | 97.9 | 51.6 |
| 6 | Stock | 5000 | 99 | 12.5 | 10.4 | 98.3 | 50.5 |
| 7 | 502 | 0 | 30 | | | 25.6 | 28.2 |
| 8 | 502 | 0 | 18 | 11.5 | 0.9 | 19.5 | 25.4 |
| 9 | 502 | 0 | 27 | | | 19.0 | 21.1 |
| 10 | 502 | 5000 | 29 | | | 30.7 | 31.2 |
| 11 | 502 | 5000 | 43 | 14.7 | 3.0 | 35.1 | 32.6 |
| 12 | 502 | 5000 | 50 | | | 34.9 | 30.6 |
| Averages of all animals receiving beryllium | | | | | | | |
| NUMBER OF RATS | | | | | | | |
| 16 | 502 | 0 | 22.9 | 11.4 | 2.5 | 21.3 | 25.5 |
| 15 | 502 | 5000 | 37.1 | 14.1 | 3.5 | 30.7 | 31.8 |

* International units of vitamin D as irradiated ergosterol given per animal per day.

diet with those on the beryllium-containing diet it is clear that the addition of the metal greatly decreased the rate of growth. The rate on the latter diet, however, was increased slightly (statistically significant) by the administration of irradiated ergosterol. Also, the giving of the vitamin D supplement to the animals on the beryllium-containing diet produced a slight rise in serum calcium and a considerable rise in phosphate. The average of the serum phosphate for the animals not receiving vitamin D on the beryllium diet is probably somewhat higher than the true value as just previous to the end of the experiment several of these animals were

consuming but little food and consequently losing weight. Under these conditions inanition invariably leads to an increase in the inorganic phosphate of the serum.

Apparently the increased serum phosphate in the animals receiving vitamin D was reflected in the degree of calcification of the bones. Although still far below those on the stock diet the absolute and relative amounts of femur-ash of the beryllium plus vitamin fed animals were definitely greater than those of the animals receiving only beryllium. Clinical examination of the wrists previous to killing the animals showed a marked difference between these 2 groups. The wrists of the rats not receiving the vitamin were much enlarged while the others appeared normal. Examination by the line test showed a wide uncalcified area at the metaphyses of the bones of the animals receiving no irradiated ergosterol, whereas this area appeared practically normal in the vitamin D fed animals. Similar results in all respects were obtained with 22 animals which were fed aluminum sulfate and with 6 animals given strontium carbonate.

In addition to the above experiments the effect of increasing the daily dose of vitamin D and the length of time it was given were studied. Although the larger amounts of irradiated ergosterol considerably increased the levels of calcium and phosphate in the serum the amount of calcification was still definitely below normal. Thus the averages for 3 rats which received, in addition to diet 502, 25000 I. U. of vitamin D per day for a period of 6 weeks were as follows: Serum calcium—15.6 mgm.; serum phosphorus—7.6 mgm.; femur ash—43.8 mgm. and 37.1 per cent. From these data it appears that large amounts of vitamin D can increase the calcification at the metaphyses when a diet which contains a metal which forms an insoluble phosphate is being fed. Furthermore, since there is an increase in both the absolute and relative amounts of bone ash it would seem that at least part of the phosphorus concerned with this calcification has come from the food. However, even when amounts of vitamin D only slightly below the toxic level are given the rate of ossification is far below normal. It is impossible to say if the vitamin increases the absorption of the phosphorus under these conditions or assists in a more economical utilization of that which is absorbed. In any case these experiments fail to determine if in times of phosphorus-want there can be a transfer of phosphorus from one part of the bone to another as appears to be true for calcium.

*B. Influence of the addition of metals which form an insoluble phosphate on the toxicity of irradiated ergosterol.* Numerous reports have appeared concerning the relation of the amounts of phosphorus in the diet to the toxicity of irradiated ergosterol. If the toxicity of large doses of vitamin D can be augmented by increasing the phosphorus of the diet, then it might

be possible to decrease the toxicity by making the phosphorus which is there unavailable. With this in mind the toxicity of irradiated ergosterol was compared with and without the addition of metal. Aluminum sulfate, aluminum acetate and beryllium carbonate were each used for the added salt, and the activated ergosterol was given in daily doses of 2000, 2500, 3000 and 5000 I. U. per 10 grams of body weight. In all, about 120 adult rats were employed in these studies. The approximate minimum lethal dose and the survival time on various levels of fatal doses were determined. There was no consistent difference in any case between the animals receiving one of the salts and the controls on the stock diet. The M.L.D. was found to be between 2000 and 2500 I. U. per 10 grams of body weight. The serum calcium and phosphate of a number of animals which had received 2000 I. U. per 10 grams of body weight for a period of 3 months on the stock diet were compared with similarly treated animals on diet 502 (stock + 1.0 per cent $BeCO_3$). The averages for 6 animals on each of these diets were respectively: calcium 14.5 and 15.0; phosphorus 5.5 and 8.1. It is apparent that there was no difference in the levels of calcium but the phosphate of the animals receiving the beryllium appears to have been definitely below the controls. However, in another experiment conducted in a similar manner but for only two weeks instead of three months there was no difference in either the calcium or the phosphate of the serum of the animals fed ration 502 and those fed the stock diet. Ash determinations on the femurs of the animals which were on experiment for three months indicated that there had been some loss of bone salts during this time, but there was no difference in this respect between the animals receiving both beryllium and irradiated ergosterol and those receiving only the vitamin preparation. The average for each group was 52.1 and 52.6 per cent which compares with a normal of about 60 per cent. Apparently in these experiments the toxicity of irradiated ergosterol was not reduced by making the phosphorus of the diet unavailable by adding to the diet metals which form insoluble phosphates.

*C. Effect of change in serum phosphate on serum calcium.* There is considerable evidence to indicate that under certain conditions there is a reciprocal relation between serum calcium and inorganic phosphate. However, it appears that this is not always the case. In a previous publication (13) this subject was discussed, and it was stated, without presenting the data, that it was possible to materially decrease the level of serum inorganic phosphate by feeding aluminum salts with little or no rise in calcium. Since then these experiments have been repeated using beryllium carbonate as the salt. Fourteen adult female rats were taken from the regular stock colony. Two were bled as controls and the remaining 12 were given ration 502. Two were sacrificed at weekly intervals and the serum analyzed for calcium and phosphate. The results, which

are presented in table 2, show clearly that it is possible for the inorganic phosphate of the serum to fall to very low levels without any detectable rise in serum calcium.

It has been frequently observed that if young animals are placed on a high calcium-low phosphorus rachitogenic diet for some time and then transferred to a similar diet from which the added calcium has been omitted there is a precipitous fall in calcium and a pronounced rise in the inorganic phosphate of the serum. Frequently this change in the composition of the serum is accompanied by tetany. In these cases the most obvious change in the diet is a reduction in the amount of calcium. However, the decrease in calcium has the effect of increasing the amount of phosphorus

TABLE 2

*Influence of lowered serum phosphate on serum calcium*

| RAT NUMBER | TIME ON DIET* | SERUM | |
|---|---|---|---|
| | | Calcium | $PO_4$-P |
| | *weeks* | *mgm. per 100 cc.* | *mgm. per 100 cc.* |
| 13 | 0 | 11.3 | 5.6 |
| 14 | 0 | 10.9 | 5.9 |
| 15 | 1 | 11.2 | 1.6 |
| 16 | 1 | 11.5 | 1.6 |
| 17 | 2 | 11.5 | 1.5 |
| 18 | 2 | 11.3 | 1.3 |
| 19 | 3 | 11.1 | 1.1 |
| 20 | 3 | 10.9 | 1.4 |
| 21 | 4 | 9.9 | 1.1 |
| 22 | 4 | 11.0 | 1.4 |
| 23 | 5 | 10.7 | 1.0 |
| 24 | 5 | 10.9 | 0.9 |
| 25 | 6 | 10.9 | 0.5 |
| 26 | 6 | 11.8 | 0.9 |

* Stock ration + 1.0 per cent beryllium carbonate.

as a larger portion of that present is absorbed. Thus it is difficult to conclude if the change in the blood is due to a lowered intake of calcium, an increase in the absorbed phosphorus or both. By adding beryllium carbonate to a high calcium-low phosphorus rachitogenic diet such as the Steenbock-Black diet 2965 (14) it would be possible to reduce the calcium after the development of rickets without increasing the availability of the phosphorus. When such a diet was fed to young rats it was found possible to suddenly omit the added calcium carbonate without any appreciable change in the levels of serum calcium and phosphorus and consequently with no indications of tetany.

In another series of experiments aluminum salts, either the sulfate or

acetate, were fed in addition to the stock diet for a period of approximately 3 weeks. The metal was then omitted from the diet. Of 22 young rats so treated a few were observed in tetany, and invariably there was a pronounced rise in phosphorus and a definite drop in calcium. Thus it appears that under these conditions the primary effect is on phosphate and not on calcium. As stated above usually it is necessary to work with young animals and to keep them on the high calcium-low phosphorus diet for some time before the change is made if tetany is to result. During this time rickets develops and when the phosphate of the serum is increased there is a rapid deposition of calcium phosphate in the rachitic bones. The calcium is drawn so rapidly from the blood that it is impossible to maintain the normal level. It seems that a sudden increase in serum phosphate coupled with a rachitic condition is the primary factor in the production of the hypocalcemia and the tetany which frequently follows.

*D. Effect of aluminum sulfate on the femur-ash content of adult rats.* As metals which form an insoluble phosphate can prevent calcification by producing a type of phosphorus deficiency, it is possible that under similar circumstances bones of adult rats might show a loss of mineral constituents. To determine to what extent this may take place 6 adult female rats were placed on the stock diet referred to in section A to which was added 5 per cent $Al_2(SO_4)_3 \cdot 18H_2O$. Six female rats of approximately similar age and history were given the stock diet without the aluminum salt. Three months later when the animals were killed the sera gave the following averages for calcium and inorganic phosphorus: aluminum-fed animals 11.8 and 2.3 and control animals 12.6 and 4.7 mgm. per cent respectively. The averages for the femur ash of the rats receiving the aluminum were 146 mgm. and 53.5 per cent and for the controls the values were 223 mgm. and 62.0 per cent. It is clear that the aluminum sulfate produced a definite decrease in the amount of mineral constituents in the bones. This was in all probability due to the marked decrease in serum phosphate which tended to shift the inorganic salts from the bone to the blood. Here again, however, there was no increase in the level of serum calcium in spite of the fact that considerable quantities of calcium must have passed from the bones into the blood. Judging from the comparative amounts of ash in the femurs of each group at least one-third of the ash constituents of the skeleton were lost during the three-month period in which the aluminum sulfate was being given.

## SUMMARY

Four per cent basic aluminum acetate, 5 per cent $Al_2(SO_4)_3 \cdot 18H_2O$, 1 per cent $BeCO_3$ and 3 per cent $SrCO_3$ added to a stock diet were each found to produce a marked rickets in rats with an accompanying low serum phosphate. Large doses of irradiated ergosterol either prevented or cured

the rachitic condition as determined by calcification at the metaphyses of the long bones. The inorganic phosphate of the serum was increased as were also the absolute amount and percentage of femur ash. Doses of irradiated ergosterol slightly below the toxic level, however, failed to produce normal calcification as compared to animals on the stock diet.

The addition to the diet of metals which form an insoluble phosphate did not decrease the toxicity of large doses of irradiated ergosterol.

The presence of 1 per cent of $BeCO_3$ in the diet of adult rats produced a very low level of inorganic phosphate in the serum within one week. The serum calcium failed to show a reciprocal rise at any time during the period of 6 weeks while the phosphorus remained at a concentration of approximately 1 milligram per 100 cc. If beryllium or aluminum salts were added to a high calcium-low phosphorus rickets-producing diet and the diet fed until rickets developed the calcium could be dropped out without any material change in the calcium and phosphate levels of the blood and without the occurrence of tetany.

When adult rats were placed on a diet containing 5 per cent aluminum sulfate for a period of 3 months there was a marked decrease in serum phosphate and, judging from ash analyses of the femurs, nearly $\frac{1}{3}$ of the mineral constituents of the skeleton were lost. There was no rise in serum calcium.

## REFERENCES

(1) Stoeltzner, H. Biochem. Ztschr. **12:** 119, 1908.
(2) Lehnerdt, F. Beitr. path. Anat. **46:** 468, 1909; **47:** 215, 1910.
(3) Shipley, P. G., E. A. Park, E. V. McCollum, N. Simmonds and E. M. Kinney. Bull. Johns Hopkins Hosp. **33:** 216, 1922.
(4) Hess, A. F. Rickets including osteomalacia and tetany, p. 81. Lea and Febiger, Philadelphia, 1929.
(5) Guyatt, B. L., H. D. Kay and H. D. Branion. J. Nutrition **6:** 313, 1933.
(6) Jones, J. H. J. Biol. Chem. **111:** 155, 1935.
(7) Deobald, H. J. and C. A. Elvehjem. This Journal **111:** 118, 1935.
(8) Sobel, A. E., A. R. Goldfarb and B. Kramer. J. Biol. Chem. **108:** 395, 1935.
(9) Jones, J. H. and B. N. E. Cohn. J. Nutrition **11:** 293, 1936.
(10) Steenbock, H. Science **58:** 449, 1923.
(11) Clark, E. P. and J. B. Collip. J. Biol. Chem. **63:** 461, 1925.
(12) Gunther, L. and D. M. Greenberg. J. Biol. Chem. **82:** 551, 1929.
(13) Jones, J. H. J. Biol. Chem. **115:** 371, 1936.
(14) Steenbock, H. and A. Black. J. Biol. Chem. **64:** 263, 1925.

# REFLEX HYPERPNEA AND VASOCONSTRICTION DUE TO ISCHEMIC EXCITATION OF THE CAROTID BODY[1]

CLAUDE V. WINDER, THEODORE BERNTHAL AND WILLIAM F. WEEKS

*From the University of Michigan, Ann Arbor*

Received for publication July 13, 1938

A comprehensive concept of chemical respiratory and circulatory regulation must include peripheral chemoreceptors. Starling's *Physiology* (1933) printed the prediction that an extension of Gesell's (1925, 1929) theory of chemical control of respiration to the recently discovered carotid chemoreceptors would probably still explain known facts. The extension is receiving a growing body of factual support. The epithelioid cells of the carotid body, on anatomical bases presumably the specific receptor cells (de Castro, 1927–1928), have an internal structure suggestive of an active metabolism (de Castro, 1926). Since they have a relatively high volume to surface ratio, they may be more susceptible than the common cell to an inadequate environment in relation to their own metabolism. A necessary link in their excitation by anoxia appears to be connected with the formation of intermediate or final acid products of anaerobic glycolysis (Winder, 1937). Their degree of excitation in general parallels their theoretical internal acidity under a variety of conditions (Bernthal, 1938). When their mechanism for anaerobic glycolysis is disrupted, they are as prone to depression by anoxia (Winder, 1937) as the central portion of the respiratory machinery (cf. Gesell, 1938). As by anoxia, so by cyanide, their reflex excitatory influence is absent when anaerobic glycolysis is suppressed and this holds as well for the influence on blood pressure as on breathing (Winder, unpublished results). When their rate of metabolism is increased relative to the adequacy of their environment by a local rise in temperature, their degree of excitation increases and *vice versa* (Bernthal and Weeks, 1938).

If, as such evidence indicates, the state of excitation of the chemoreceptors is a product of interaction of their own acid metabolism with their immediate environment rather than *primarily* a function of the chemical quality of the environment *per se*, then failure of refreshment of the environment should lead to a cumulative disturbance resulting in excitation. That is, a purely quantitative anemia of the chemoreceptors without

[1] These experiments were supported in part by a grant from the Rockefeller Foundation to Robert Gesell for studies on respiration.

exogenous change in the quality of the blood should reflexly excite respiration and cardiovascular changes. The present experiments test whether this additional requirement of the hypothesis is to be satisfied.

Bogue and Stella (1935) and Samaan and Stella (1935) observed that gross cephalic or generalized anemia caused the appearance of a chemoreceptive discharge from the carotid body. Presumably these impulses would pass in the afferent limb of a reflex arc which normally excites respiratory and cardiovascular changes. But at present a direct demonstration of such reflex effects in experiments fulfilling the triple requirement of effective *local* ischemia, exclusion of known pressoreceptors from the attendant pressure change and an intact reflex mechanism appears to be lacking.

Effective local ischemia is established only with some difficulty. In the dog with intact cephalic arteries there are free arterial anastomoses between the carotid system, the normal supply to the carotid body, and other cephalic arteries, notably the vertebral system (Cooper, 1836; Ellenberger and Baum, 1891), which may vicariously and most efficiently circulate the carotid body when intracarotid pressure is somewhat reduced (Winder, 1933), even though conveniently accessible efferent carotid vessels be tied (Comroe and Schmidt, 1938; Winder, 1938). In view of these anastomoses, negative results of common carotid occlusion (Dautrebande, 1931; Bouckaert, Dautrebande and Heymans, 1931; Camus, Bénard and Merklen, 1934) or of marked changes in perfusion pressure (Comroe and Schmidt, 1938), after pressoreceptor exclusion, do not negate ischemic excitation of the carotid body. Conversely, positive results from experiments in which establishment of effective ischemia is equally improbable, but in which *pressoreceptors* are *left intact*, hardly justify the conclusion on indirect grounds (von Euler and Liljestrand, 1936, 1937) that the hyperventilation attending lowered intracarotid pressure is due to anemic *chemical* stimulation. Further, experiments show (Winder, 1938) that perfusion of incompletely isolated carotid bifurcations with an unnatural perfusion fluid (von Euler and Liljestrand, 1937) introduces serious complications due to alternate exposures of the carotid bodies to natural blood and unnatural perfusing fluid when relative values of perfusion and systemic blood pressures are experimentally reversed.

In spite of these difficulties in the way of a direct experiment there is strong indirect evidence that one of the reflex changes in question may result from carotid body ischemia. In the decerebrate cat, where decerebration was believed to have interfered with the normal vertebral-carotid anastomoses, Stella (1936) found an unusually intense respiratory response to common carotid occlusion, and the indirect evidence presented that it was largely due to interference with chemoceptor circulation seems in part acceptable. Winder (1938) while comparing responses to intra-

carotid pressure changes in two groups of experiments, in one of which the carotid body circulation was left patent and in the other of which it was excluded by embolization, observed in the former group marked changes in breathing movements from pressure changes within a small range over zero (cf. Schmidt, 1932), that is, in a range below the threshold for the independent sinus stretch reflex studied in the second group. The isolation of the bifurcation in the first group must have eliminated the anastomoses so that at near-zero perfusion pressures anemic excitation of the carotid body occurred. Rudberg (1938) observed in the cat and rabbit that when the general blood-pressure was lowered below a certain critical level, the increase in breathing attending carotid occlusion was considerably augmented.

In the present experiments the reflex end-effects themselves were recorded. Effective carotid body ischemia was insured by appropriate dissections and ligations, to be described. With reference to exclusion of pressoreceptors, histological studies have failed to show pressoreceptors in the carotid walls above or below the sinus proper (de Castro, 1927–1928; Sunder-Plassman, 1930). Consequently, Gollwitzer-Meier's (1934) ligature 6 placed around the external carotid artery between the origins of the carotid sinus and the occipital artery may be depended upon to separate known pressoreceptors from the carotid body circulation. Comroe and Schmidt (1938) employed that means of separation when they found that pressure changes of 200 mm. Hg on the carotid body side of the ligature were ineffective, and thus provided physiological evidence that pressoreceptors are excluded by the ligature from the carotid body segment. This finding, together with the afore-mentioned negative results from *common carotid occlusion* after restricted denervation of the sinus and bifurcation, indicates not only that endings in the vessels of the carotid body tentatively described by de Castro (1926; 1927–1928) as lesser pressoreceptors are not visibly effective as such, but also that chemoreceptors do not respond to pressure changes *per se*. More directly, the electrical studies by Bogue and Stella (1935) and Samaan and Stella (1935) indicate that pressure changes as such do not result in altered discharge from any receptors in the carotid body.

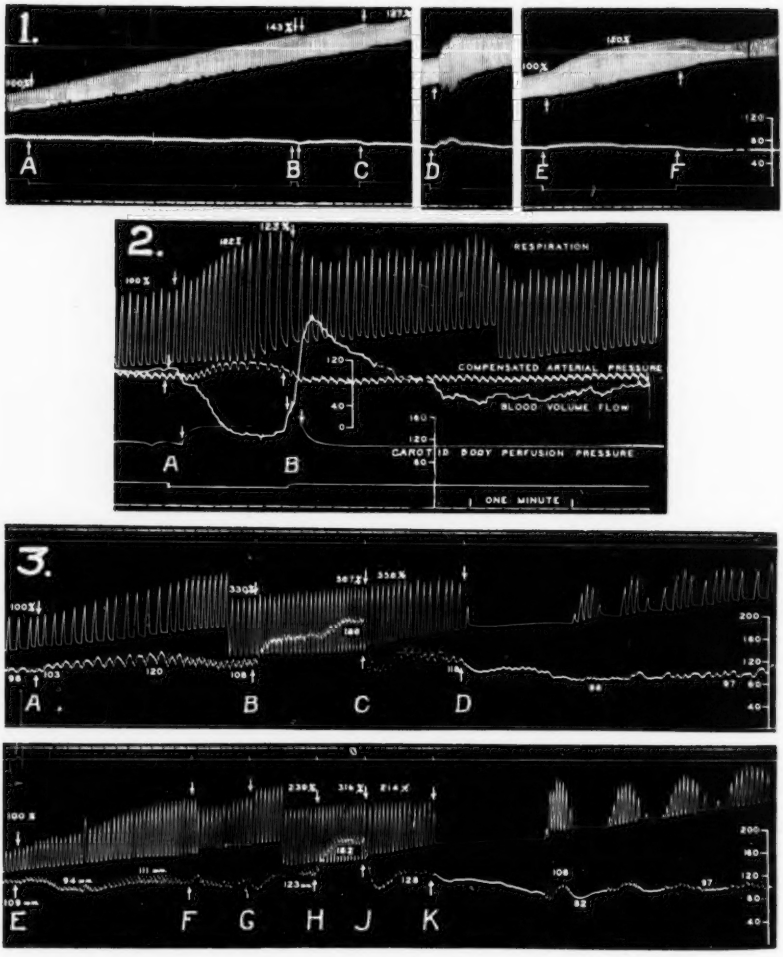
We have now made 19 tests for ischemic excitation in 5 dogs and with uniformly positive results. The technique varied in details. In the experiments from which figures 1 and 2 are taken, arterial connections between the carotid bifurcations and surrounding tissues were sought out by transillumination and tied individually. Absence of back-flow into either bifurcation over a period of several minutes when clamped off and emptied of blood indicated completeness of arterial isolation. The vago-sympathetic-aortic nerves were severed. In the case of figure 1, a ligature was placed about the origin of the carotid sinus on one side (a procedure

which Gollwitzer-Meier, 1934, found in almost all cases to abolish the pressure responses) and the external carotid stripped as far as the origin of the occipital artery. On the other side, with fine forceps and a fine aneurism needle Gollwitzer-Meier's ligature 6 was worked around the external carotid close to its wall and with minimal disturbance of the intercarotid tissue, and tied. The animal was heparinized and artificial vascular connections made between the stumps of the two external carotids. The two occipital arteries were cannulated and their outflow led into an axillary vein. Blood flowing through the one common carotid thus supplied both carotid bodies exclusively.

The gradually elicited hyperpnea due to occlusion of the patent common carotid is shown in figure 1 *A*. A momentary de-occlusion at *B* caused an immediate reduction in hyperpnea, illustrating a trigger-like sensitivity to an abruptly refreshed blood supply. De-occlusion was maintained at *C*. At *D*, 1.0 cc. of 0.01 M sodium cyanide was injected into the patent common carotid. Among other things, the cyanide may have caused severe reduction in intracellular buffer efficiency by virtue of anaerobic acid-forming metabolism and it is interesting that shortly thereafter repetition of occlusion at *E* produced a much more immediate ischemic response than previously, as though the receptor cells were metabolizing on a lesser margin of environmental adequacy relative to their altered state.

Figure 2 demonstrates vasomotor as well as respiratory evidence of carotid body ischemic excitation. Both carotid bifurcations were arterially isolated as before, ligature 6 tied on both external carotids the vago-sympathetic-aortic nerves severed and the animal heparinized. While maintaining constant conditions on the sinus sides of the segregating ligatures, the carotid body sides were perfused with normal arterial blood through external carotid (in), occipital artery (out), axillary vein circuits. Upon establishing carotid body ischemia by interruption of perfusion (*A* to *B*) the usual hyperpnea was accompanied by blood volume flow changes in the foreleg indicative of marked reflex vasoconstriction similar to that observed from various other types of carotid body stimulation (Bernthal, 1938).

In the experiment from which figure 3 is taken, the vago-sympathetic-aortic nerves were severed and one carotid bifurcation denervated. The major efferent vessels of the other bifurcation except the lingual artery were tied individually. Ligature 6 was worked around the external carotid. At this stage, very carefully but firmly tightening the ligature for several minutes was without obvious effect on circulation or respiration, indicating: *a*, accordance with foregoing evidence that there are no effective pressoreceptors beyond such a ligature; *b*, that manipulative consequences of tightening the ligature were absent, and *c*, that if ischemic carotid body




Figs. 1-3. Dogs. Morphine and chloralose. "Vagotomized." Respiration recorded by means of spirometer connected with rebreathing circuit. Arterial and carotid body perfusion (fig. 2) pressures by means of mercury manometers. (In fig. 2 occlusion *A B* is distal to the recording manometer.) Volume flow of blood (fig. 2) in right axillary artery recorded thermo-electrically. See text for details of preparation and experimental procedures.

excitation is possible, the carotid body was being vicariously circulated through arterial anastomoses. The bifurcation was then *completely* isolated arterially, i.e., all remaining connections with surrounding tissue

were tied off with fine mass ligatures, excepting only the previously exposed sinus nerve and a small vein which appeared to be draining the intercarotid tissue and possibly the carotid body. Under these conditions, tying the ligature (fig. 3 *A* or *E*) was followed by gradually developing hyperpnea and gradual and slight, but definite, rise in arterial blood pressure in spite of the action of that rise on the intact carotid sinus, the inevitable acapnia to which the centers were exposed and a certain unknown degree of recurrent pressure through the lingual artery. The response must have been due to carotid body ischemia. Disregarding for the moment the intermediate procedures, abruptly reëstablishing effective carotid body circulation at *D* or *K* by carefully cutting the ligature was followed immediately by apnea and hypotension, with recovery characterized by well marked periodicity. It is interesting that the apnea and subsequent periodicity appear identical to those frequently seen on abrupt readministration of room air after prolonged intrapulmonary low $O_2$ gas administration, or after prolonged forced breathing. They are supplementary indications of the reflex strength of the ischemic excitation.

The relatively gradual onset of carotid body ischemic excitation and its eventual intensity appear to tell a tale of cumulative influence of persistent interaction between cellular metabolism and laggingly refreshed or static environment. The abrupt subsidence on suddenly refreshing the environment indicates a delicately sensitive mechanism.

It is apparent that the respiratory reactions to carotid sinus pressoreceptive disinhibition and to carotid body ischemic excitation occur in the same direction and this would tend toward experimental confusion of one with the other. In fact, von Euler and Liljestrand (1936, 1937), assuming upon questionable grounds that the hyperpnea attending diminished intracarotid pressure is due to chemical stimulation, have stated that "no evidence has been obtained that a direct inhibitory influence on respiration is exercised by the arterial blood pressure itself on the sinus region." Inasmuch as the present experiments necessitated *anatomical* segregation of pressoreceptors from chemoreceptors, they provided a unique opportunity for *functional* segregation as well, and simultaneous demonstration of the two reactions as entirely independent albeit externally similar phenomena.

Such demonstration is illustrated by the intermediate procedures in figure 3. Carotid body ischemic hyperpnea had become well established (*A* or *E*, et seq.) while the pressoreceptors were left exposed to systemic arterial pressure. The endosinual pressure was then dropped by unclamping the superior thyroid artery to allow escape of blood through an incision peripheral to the clamp and then immediately occluding the common carotid (fig. 3 *B*). The familiar vasomotor disinhibition is most obvious and accompanying it is a well marked incremental hyperpnea. Repeated

at *F*, the response was complicated by violent micturition, but at *H* the added hyperpnea was again demonstrated. Inasmuch as there are no known chemoreceptors in the carotid sinus, *this superimposed hyperpnea must have originated from pressoreceptive disinhibition.* The transiently greater intensity of reversal of the pressoreceptive responses at *C* and *J* are to be expected from an abrupt exposure of the sinus to a momentarily greater blood pressure.

It is of some importance that the hyperpnea elicited by (submaximal?) ischemic excitation of a single carotid body (e.g., 230 per cent increase) may greatly exceed the largest response observed upon reducing endosinual pressure (from systemic arterial, e.g., 123 mm. Hg, fig. 3 *H*) to subthreshold at a single carotid sinus either in these experiments (72 per cent) or in those reported previously (Winder, 1938) when chemoreceptors were excluded by carotid body embolization (104 per cent increase due to pressoreceptive reflex *dis*inhibition). This may be regarded as added evidence that the ischemic response is truly independent of any pressoreceptors which may possibly have remained on the carotid body side of the separating ligature.

## SUMMARY

In anesthetized dogs carotid body chemoreceptors were vascularly segregated from carotid sinus pressoreceptors by means of *complete* arterial isolation of the bifurcation and the use of suitable ligatures. Carotid body ischemia caused marked chemoreceptor excitation as evidenced by reflex hyperpnea, peripheral vasoconstriction and arterial hypertension.

The hyperpnea and vasoconstriction thus elicited were shown to be separate from the qualitatively similar end-actions demonstrated to result from pressoreceptive disinhibition.

Excitability of the chemoreceptors by ischemia meets a requirement of the hypothesis that in general their state of excitation is a product of the interaction of their own acid metabolism with their immediate environment.

## REFERENCES

BERNTHAL, T. G. This Journal **121:** 1, 1938.

BERNTHAL, T. G. AND W. F. WEEKS. Proc. Am. Physiol. Soc., Baltimore, 1938; full report in preparation.

BOGUE, J. Y. AND G. STELLA. J. Physiol. **83:** 459, 1935.

BOUCKAERT, J. J., L. DAUTREBANDE AND C. HEYMANS. Ann. de physiol. **7:** 207, 1931.

CAMUS, L., H. BÉNARD AND F. P. MERKLEN. Compt. rend. Soc. de biol. **115:** 1626, 1934.

DE CASTRO, F. Trav. du lab. recherch. biol. de l'Univ. de Madrid **24:** 365, 1926. Ibid. **25:** 331, 1927–1928.

COMROE, J. H., JR. AND C. F. SCHMIDT. This Journal **121:** 75, 1938.

COOPER, A. Guy's Hosp. Reports **1:** 457, 1836.
DAUTREBANDE, L. Médecine **12:** 694, 1931.
ELLENBERGER, W. AND H. BAUM. Anatomie des Hundes. Berlin, 1891.
VON EULER, U. S. AND G. LILJESTRAND. Skand. Arch. f. Physiol. **74:** 101, 1936.
Ibid. **77:** 191, 1937.
GESELL, R. Physiol. Rev. **5:** 551, 1925.
Ergebn. d. Physiol. **28:** 340, 1929.
Internat. Physiol. Congr., Zurich, 1938.
GOLLWITZER-MEIER, K. Pflüger's Arch. **234:** 342, 1934.
RUDBERG, T. Skand. Arch. f. Physiol. **79:** 8, 1938.
SAMAAN, A. AND G. STELLA. J. Physiol. **85:** 309, 1935.
SCHMIDT, C. F. This Journal **102:** 94, 1932.
STARLING, E. H. AND C. L. EVANS. Principles of human physiology. Philadelphia, 1933.
STELLA, G. Arch. internat. de pharmacod. et de thérap. **53:** 289, 1936.
SUNDER-PLASSMANN, P. Ztschr. f. Anat. u Entwcklungsgesch. **93:** 567, 1930.
WINDER, C. V. This Journal, **106:** 28, 1933.
Ibid. **118:** 389, 1937.
Ibid. **122:** 306, 1938.

# RESPIRATORY EXCHANGE DURING HIGH CARBOHYDRATE INGESTION[1]

JOHN H. TALBOTT, F. S. COOMBS, W. V. CONSOLAZIO AND L. J. PECORA

*From the Medical Clinic of the Massachusetts General Hospital and the Fatigue Laboratory, Harvard University, Boston*

Received for publication June 20, 1938

In non-diabetic subjects, the ingestion of large amounts of simple sugars is associated with an elevation above unity of the respiratory quotient (R.Q.), a sparing action on protein metabolism, and under certain conditions, an increased tolerance for sugar as determined by successive glucose tolerance curves. Most of the data from which these deductions have been drawn were obtained during acute experiments on animal or human subjects. The duration of the high carbohydrate ingestion generally has been less than a week; an adequate definition of the physiological effects of longer periods of ingestion has not been derived. The experiments to be described in this communication indicate that when a regime of excessive sugar ingestion is maintained for from 14 to 48 days, the R.Q. in the morning before breakfast may remain above unity for 2 or more weeks. Within the time limits of these experiments, the carbohydrate-fat conversion processes do not reach a steady state.

EXPERIMENTS. Five adult males, asthenic in appearance, were observed from 2 to 7 weeks on a diet (table 1) adequate with respect to protein, fat, minerals and vitamins and fortified by the addition daily of from 430 to 700 grams of sucrose, glucose or levulose.[2] The subjects were selected from the relief rolls of the City of Boston and on admission were thought to be suffering only from undernutrition. All the data were collected in the metabolism ward of the Massachusetts General Hospital,

[1] This investigation was aided by the Corn Industries Research Foundation.

[2] The caloric value of the individual sugars was assumed to be the same (Mitchell, Hamilton and Beadles, 1937) and the degree of purity of the added sugars to be constant during the individual observation periods. Commercial sucrose was obtained from the Revere Sugar Company. Polariscopic examinations of several batches of sugar while these experiments were being done showed a concentration in each sample that was greater than 99.4 per cent. Glucose, which was obtained in powder form from the Corn Products Refining Company, contained about 8 per cent by weight of water on analysis. In the summation of glucose consumption per day this correction has been applied. Levulose, C. P., was obtained from Pfanstiehl and Company and was reputed to be more than 99 per cent pure.

where the subjects were ambulatory through the day and received approximately nine hours of sleep each night.

The respiratory quotient and oxygen consumption were determined approximately every third day during the respective observation periods. A 600 liter gasometer was used for collecting expired air by the open system. Each determination was begun approximately 16 hours after the evening meal of the previous day and 12 hours after the last ingestion of sugar solution. It was continued for 50 minutes in order to minimize any transient variations in the respiratory quotient which may be observed in 10 minute periods of collection.

After the respiratory exchange had been measured, a sample of capillary blood for determination of blood sugar concentration was drawn. The subject who received levulose as the principal sugar was given 100 grams of

TABLE 1

*Experimental observations during high carbohydrate ingestion*

| SUBJECT | INITIAL BODY WEIGHT | TIME | MAXIMUM FASTING BLOOD SUGAR | DAILY INTAKE | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Calories | Protein | Fat | Carbohydrate with meals | Added carbohydrate |
| | *kgm.* | *days* | *mgm. per cent* | | *grams* | *grams* | *grams* | *grams* |
| Wo. | 58.7 | 1 | 70 | 2300 | 67 | 50 | 75 | 316 |
| | | 2–10 | 84 | 3030 | 72 | 51 | 114 | 462 |
| | | 11–31 | 88 | 3560 | 76 | 60 | 148 | 530 |
| | | 32–48 | 90 | 4860 | 106 | 151 | 259 | 510 |
| Wi. | 62.4 | 1–39 | 96 | 4040 | 76 | 60 | 208 | 590 |
| Mo. | 44.6 | 1–47 | 118 | 4340 | 78 | 97 | 152 | 638 |
| Pu. | 55.5 | 1–12 | 102 | 4300 | 74 | 97 | 152 | 638 |
| Je. | 50.4 | 1–40 | 120 | 3550 | 78 | 54 | 188 | 500 |

glucose following measurement of the metabolic rate. Capillary blood samples for the blood sugar determinations were then obtained every half-hour for the following three hours. Urinary nitrogen studies during a portion of the observation period were carried out on one subject. The method of Benedict (1911) was used for detection of reducing substances in the urine. The methods for the analysis of the blood sugar and urinary nitrogen have been described previously by Talbott, Jacobson and Oberg (1935).

RESULTS. The data of the post-absorptive R.Q.'s which were collected in the morning before breakfast are shown in figure 1. There was a rise above unity of this function following ingestion of each of the three sugars. The maximum elevation was observed in the two sucrose experiments. In Pu. the R.Q. was 1.21 on the 5th day, which decreased to 1.09 on the

9th day. In Mo. this function reached 1.22 on the 6th day, remained above unity until the 20th day, and decreased only to 0.96 on the 45th day of the experiment. In the two subjects who received glucose as the added sugar, Wo. and Wi., the R.Q.'s, which were quantitatively similar, reached or exceeded 1.05 by the 9th day and remained above unity during an elapsed time of approximately 3 weeks. In the second half of the observation period, the R.Q.'s were of the same order of magnitude as in the sucrose experiment. One subject only, Je., was given levulose. His response was unlike that which followed either glucose or sucrose ingestion. In this experiment the R.Q. was 1.05 on the 8th day, but decreased to 0.96 two days later and remained below that observed for the other sugars throughout the remainder of the observation period.

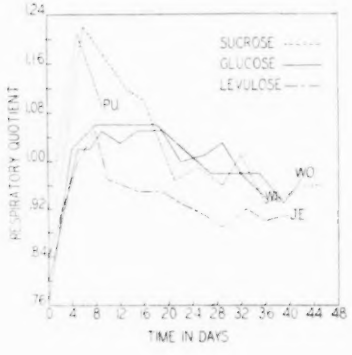



Fig. 1

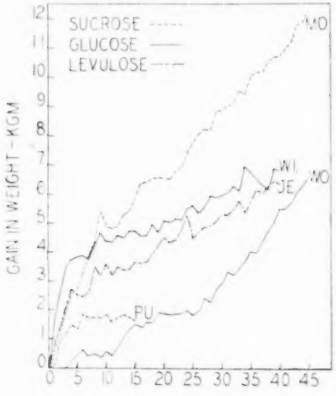



Fig. 2

Fig. 1. The post-absorptive respiratory quotient during high carbohydrate ingestion.

Fig. 2. Gain in body weight during high carbohydrate ingestion.

The non-protein respiratory quotients were calculated for all of the experiments and were from 0.03 to 0.17 greater than the uncorrected ones. With the correction applied, the general nature of the response was not altered, although in the glucose and sucrose experiments, the number of mornings on which the R.Q. exceeded unity was increased.

The consumption of oxygen was below normal on admission in all subjects but Je. In Wo. and Wi., the function remained below normal during the respective periods of observation, while in Mo. it increased to normal during the first three weeks.

The data for gain in body weight are shown in figure 2. There appeared to be no obvious correlation between gain in weight and ingestion of any particular sugar.

The persistence of the elevation of the R.Q. during a fast preceded by a consistently high carbohydrate intake, was investigated in 2 subjects. In these experiments only, was the respiratory exchange studied in the absorptive as well as the post-absorptive state. These observations for Je. are shown in figure 3. On the 4th day of a high sucrose diet the respiratory exchange was determined at frequent intervals. At the beginning of the day it was 1.03 and in the evening it was 1.18. The metabolic rate also increased during the absorptive period. The subject then fasted for 36 hours. The following morning the R.Q. was 1.06. Decreasing progressively, the R.Q. was 0.84 that evening and 0.91 on the second morning. At no time during the fast was the oxygen consumption above normal.

The concentration of blood sugar in the fasting state showed little fluctuation. In Wi. and Wo. the maximum concentration of this con-

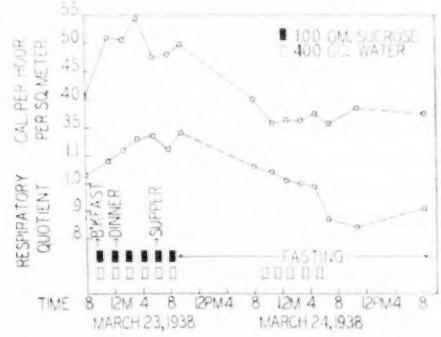



Fig. 3. The respiratory quotient and metabolic rate during high carbohydrate ingestion followed by a 36 hour fast.

stituent on the days when the R.Q.'s exceeded unity was 90 mgm. per cent. In Mo. and Pu. the maximum concentration on similar days was 104 mgm. per cent. In Je. the concentration of blood sugar on the day when the R.Q. was greatest was 120 mgm. per cent. Je. was the only subject whose tolerance of glucose was determined directly at frequent intervals. These experiments showed little variation. The small changes observed were in the direction of a diminution in tolerance.

The nitrogen excretion was investigated during two periods of 10 days each on Wo. During the first period the excretion averaged 10.1 grams per day, while during the second it averaged 8.3 grams per day. The protein intake was 72 grams per day during the first period and 76 grams per day during the second. If it is assumed that approximately 10 per cent of the total intake was excreted in the stool, there was nitrogen equi-

librium during the first 10 days. During the second period, however, the nitrogen intake was 20 per cent greater than the excretion.

Mention should be made of the absence of untoward effects from the high sugar intake. Rose (1933) has stated that gastric irritability, headache, skin eruptions and other disturbances have followed an excessively high carbohydrate diet. Neither these symptoms nor others were observed. The subjects seemed to enjoy the metabolic regime and none of them terminated the experiment prematurely. No sugar was excreted in the urine in three subjects and less than 2 grams per day was excreted in the other two (Je. and Wo.).

Discussion. In the absorptive state respiratory quotients greater than unity have been observed in man and animals by several investigators (Grafe, 1913; Magnus-Levy, 1894; Bleibtrau, 1901; Eckstein and Grafe, 1919; Carpenter and Fox, 1930 and Wierzuchowski and Ling, 1925). Our quotients were of a similar magnitude, but were determined while the subjects were fasting and were in what we have interpreted as the post-absorptive or basal state. The interpretation is based upon the following:

1. The interval between the ingestion of food and the determination of the respiratory exchange was believed to suffice for the completion of the process of absorption. Cori (1925) has observed that rats absorb about 2 grams of glucose per kilogram of body weight and that they continue to absorb sugar at a uniform rate until at least 90 per cent of the amount ingested has been absorbed. Trimble, Carey and Maddock (1933) found a uniform rate in male dogs with weights varying from 12 to 20.8 kgm., but the amount absorbed was only about 1 gram of sugar per kilogram of body weight. In our subjects, a considerably lower absorption rate would suffice to allow all of the ingested sugar to be absorbed. Thus in subject Mo. a minimum rate of 0.24 gram per kgm. of body weight would be adequate for the complete absorption of 127 grams of sucrose which were taken after the evening meal and at least 12 hours before the determination of the R.Q. In Wi. a minimum rate of 0.17 gram per kgm. would suffice. These assumptions are consistent with the conclusion of Benedict and Higgins (1912) that a "basal" R.Q. may be obtained 12 hours after the ingestion of the final portion of 600 grams of sucrose which has been consumed during the previous day.

2. The oxygen consumption which was calculated from each determination of the respiratory exchange was never greater than normal and in most instances was below normal. The effect of an increase in metabolic rate with an elevation of the R.Q. is shown for Je. in figure 3. During March 23, 1938 with an adequate diet, the oxygen consumption was from 25 to 35 per cent greater than on March 24, 1938 when the subject fasted. On the morning of the second day with a normal metabolic rate, the R.Q.

was above unity for several hours. The non-protein quotient did not fall below unity until 6:00 p.m., 22 hours after the last food was taken.

The explanation of this metabolic phenomenon is the continuous conversion of carbohydrate to fat (Lusk, 1928). Although the evidence is less convincing, it is believed that even after the R.Q. has fallen below 1.00 there is some conversion of carbohydrate to fat (Benedict, Emmes and Riche, 1911). Such an assumption is compatible with a normal or subnormal oxygen consumption for according to Lusk (1928): "The transformation of carbohydrate into fat requires the liberation of very little energy and the height of the total metabolism is scarcely affected by the process."

If the method of calculation of indirect calorimetry with respiratory quotients above unity is correct, an estimate may be made of the amount of fat formed from carbohydrate. Thus, Mo. has a maximum non-protein R.Q. of 1.39 on the 6th day of the experiment. This is equivalent to 3.5 liters of excess $CO_2$ per hour. This amount corresponds to the conversion to fat of 6.0 grams per hour (Lusk, 1915) or 3.1 kgm. in 21 days. The gain in weight during the first 21 days of the experiment was approximately twice this amount. This calculation, therefore, seems to be conservative.

There was no satisfactory correlation between the gain in body weight and the amount or kind of sugar ingested. This was illustrated by Wo. and Wi., both of whom consumed glucose. Among the five men, Wi. gained the most weight and Wo. the least. Also, during the two weeks' observation on Pu., the weight gain was quite different from that of Mo. although both had sucrose as the added sugar. The fact that all of the subjects were of the same type, i.e., asthenic, helps to rule out any individual variation in ability to put on weight.

There are at least two explanations for the varied response of the R.Q. to the several sugars. It is conceivable that the height of the elevation is an index of the ability to form fat. If this is a fact, then levulose is the poorest fat-former, glucose next, and sucrose the best. These inferences are similar to those of Fender, who investigated glycogen formation in rats following high carbohydrate ingestion. A second explanation is that the speed of conversion of sugar to glycogen to fat may vary. In acute experiments Johansson, Billström, and Heyl (1904) have shown that the rise in $CO_2$ output after levulose ingestion is greater than that following glucose ingestion. This was attributed to the fact that levulose is less readily retained as glycogen in the liver and reaches the tissues in larger amounts than does glucose and replaces fat more completely as the source of energy.

The absolute level of the R.Q. is believed to be a function of the kind of sugar ingested rather than the total amount of carbohydrate. Wi. and Mo. consumed approximately 780 grams of carbohydrate daily for more than

5 weeks while the R.Q.'s were quite different during the first 2½ weeks. On the other hand although the R.Q.'s in Wo. and Wi. were essentially similar, the amount of carbohydrate each ingested daily during the first 10 days varied by nearly 200 grams.

The change in the R.Q. during the experimental periods, with the subjects consuming a relatively constant diet, is not readily explained. Benedict and Higgins (1912) found in short experiments that "with different normal individuals the same diet will lead to essentially the same R.Q. the next morning without food." In longer experiments this statement does not appear to be valid. We would modify it so as to imply that one kind of carbohydrate will give a reproducible R.Q., but the height of the elevation changes as the experiment is prolonged.

The decrease in R.Q. after the peak of the rise may be a function of adaptation and economy of conversion of sugar to fat, although we have no additional evidence to support this hypothesis. Any excess of ingested carbohydrate must be stored as glycogen, converted into fat or excreted in the urine. The third means of disposal may be dismissed as regards these experiments. It seems unlikely that more glycogen was formed during the second half of the experimental period than during the first half. Thus, there remains the second explanation, i.e., conversion into fat. It seems reasonable to believe that in these experiments this process was not in equilibrium otherwise the R.Q.'s would have remained constant once they had reached a maximum.

## SUMMARY AND CONCLUSIONS

The effects of the ingestion of large amounts of sucrose, glucose and levulose for periods of 2 to 7 weeks were observed in 5 subjects. It produced neigher hypoglycemia, glycosuria nor untoward symptoms. The basic diet contained an adequate amount of protein, fat, minerals and vitamins. The total carbohydrate intake each day ranged from 550 to 800 grams.

The respiratory quotient was determined in the post-absorptive state at frequent intervals and values greater than unity were observed over periods as long as 3 weeks. The response of the respiratory quotient to each sugar was different. The degree of elevation was in the following order: sucrose, glucose and levulose.

## REFERENCES

BENEDICT, F. G., L. E. EMMES AND J. A. RICHE. This Journal **27:** 383, 1911.
BENEDICT, F. G. AND H. L. HIGGINS. This Journal **30:** 217, 1912.
BENEDICT, S. R. J. A. M. A. **57:** 1193, 1911.
BLEIBTRAU, M. Pflüger's Arch. **85:** 345, 1901.
CARPENTER, T. M. AND E. L. FOX. J. Nutrition **2:** 375, 389, 1930.
CORI, C. F. J. Biol. Chem. **66:** 691, 1925.

ECKSTEIN, E. AND E. GRAFE. Ztschr. Physiol. Chem. **107:** 72, 1919.
FENDER, F. A. Personal communication.
GRAFE, E. Deutsch. Arch. Klin. Med. **113:** 1, 1913.
JOHANSSON, J. E., J. BILLSTRÖM AND C. HEYL. Skand. Arch. Physiol. **16:** 263, 1904.
LUSK, G. J. Biol. Chem. **20:** 555, 1915.
The elements of the science of nutrition. Philadelphia, 4th ed., 1928.
MAGNUS-LEVY, F. Pflüger's Arch. **55:** 1, 1894.
MITCHELL, H. R., T. S. HAMILTON AND J. R. BEADLES. J. Nutrition **14:** 435, 1937.
ROSE, M. S. The foundation of nutrition. New York, 1933.
TALBOTT, J. H., B. M. JACOBSON AND S. A. OBERG. J. Clin. Invest. **14:** 411, 1935.
TRIMBLE, H. C., B. W. CAREY, JR. AND S. J. MADDOCK. J. Biol. Chem. **100:** 125, 1933.
WIERZUCHOWSKI, M. AND S. M. LING. J. Biol. Chem. **64:** 697, 1925.

# BLOOD LACTATE IN NORMAL AND SYMPATHECTOMIZED DOGS

H. T. EDWARDS[1], L. BROUHA AND R. E. JOHNSON

*From the Fatigue Laboratory, Harvard University, Morgan Hall, Boston*

Received for publication July 5, 1938

In most respects totally sympathectomized dogs act like normal dogs. For instance, they can work as long and nearly as hard on a motor-driven treadmill as they did before the operation (Brouha, Cannon and Dill, 1936), and at the same time they do not develop abnormal blood lactates. Because it now appears that in respect of blood lactate, sympathectomized dogs under some conditions react differently from normal dogs, we have investigated lactate metabolism in relation to exercise, glucose, insulin and adrenalin.

In these experiments, all carried out on dogs that had fasted for 12 to 16 hours, lactate was estimated in Folin-Wu centrifugates of the blood by the method of Friedemann, Cotonio and Shaffer (1927). All of the results are expressed as milligrams lactic acid per 100 cc. blood. In most cases blood was collected from the saphenous vein. In some of the exercise experiments we also collected blood from the heart, the external carotid and the femoral arteries. The technique of sympathectomy has been described by Brouha, Cannon and Dill (1936).

*I. Blood lactate and exercise.* In our normal dogs the resting venous blood lactate has varied between 5.4 and 14.6 mgm. per cent; in our totally sympathectomized, between 3.2 and 15.4. During exercise it rises and there is a correlation between blood lactate and both the rate of work and the room temperature (table 1).

These results are similar to those found with normal dogs by Dill, Edwards and Talbott (1932). The results with sympathectomized dogs are not produced by changes of body temperature different from those of normal dogs, because under the same conditions the rectal temperature of both normal and sympathectomized dogs varies in the same range.

Table 2 shows that the blood lactate of dogs at the end of exercise is uninfluenced by sympathectomy so long as rate and duration of work and room temperature are the same.

Dog I was tested at various times after total sympathectomy. On

[1] Died December 14, 1937.

each occasion grade of work, time of work and room temperature were the same (table 3).

Twenty-five days after the operation her lactate after running was as low as before the operation. Forty-three days after the operation she reached the same blood lactate after exercise as she did soon after the operation, but both the duration and intensity of work were greater (25 min. at 135 kgm.m./min.). This effect is perhaps related to the finding of Dulière, Bacq and Brouha (1934) that in freshly sympathectomized cat limbs, the breakdown of phosphagen during contraction is greater than normal, but in chronically sympathectomized cat limbs, it is normal. One can conclude from the present experiments that exercise has the same effect on blood lactate in normal and in chronically sympathectomized dogs.

TABLE 1

*Relation between blood lactate, room temperature and rate of work*

| DOG | CONDITION | ROOM TEMPERATURE | TIME OF RUN | RATE OF WORK | BLOOD LACTATE AT END |
|---|---|---|---|---|---|
| | | °C. | *min.* | *kgm. m/min.* | *mgm. per cent* |
| II | Chronically sympathectomized | 9 | 50 | 150 | 26.9 |
| II | Chronically sympathectomized | 19 | 36 | 150 | 39.7 |
| I | Chronically sympathectomized | 46 | 31 | 125 | 43.5 |
| I | Chronically sympathectomized | 15 | 10 | 125 | 19.3 |
| III | Normal | 6 | 112 | 212 | 12.0 |
| III | Normal | 20 | 29 | 212 | 22.5 |
| III | Normal | | 25 | 142 | 76.0 |
| III | Normal | 29 | 25 | 212 | 65.0 |
| III | Normal | 35 | 38 | 142 | 42.3 |

*II. Blood lactate after glucose ingestion.* Glucose was administered in 20 per cent aqueous solution by stomach tube in the dose of 2 grams per kgm. of body weight. Blood was drawn at intervals during the following 3 hours. In both normal and sympathectomized dogs, the blood lactate remained at its resting value throughout; under the conditions of our experiment, it is unaffected by conditions that may raise the blood sugar 100 per cent or more.

*III. Blood lactate after insulin injection.* Four units of insulin brought the blood sugar down to 32–33 mgm. per cent and caused no change in blood lactate from the resting value in normal dogs. One unit of insulin administered to sympathectomized dogs produced the same change in blood sugar and no change in lactate. This is in contrast to the results

of Scott and Berg (1936) with cats deprived of their adrenal medullae. We consider that their results do not prove an effect of insulin, since the one control experiment they reported without insulin showed a drop in blood lactate as striking as the drop after insulin injection.

*IV. Blood lactate after injection of adrenalin.* We injected 0.1 mgm. of adrenalin hydrochloride (Parke-Davis) in 0.05 per cent solution subcutaneously into both normal and sympathectomized dogs. The rise in blood lactate was much more marked in the sympathectomized than in the normal dogs (fig. 1). The curves were already separated ½ hour after injection, and the differences were greatest at 2 hours, when the maxima

TABLE 2

*Blood lactate of dogs after work in a room at 14–18°C.*

| DOG | CONDITION | RATE OF WORK | DURATION OF RUN | BLOOD LACTATE AT END |
|---|---|---|---|---|
| | | *kgm. m/min.* | *min.* | *mgm. per cent* |
| II | Normal | 150 | 50 | 25.1 |
| II | Sympathectomized | 150 | 50 | 26.9 |
| IV | Normal | 150 | 50 | 29.2 |
| IV | Sympathectomized | 150 | 50 | 29.4 |
| V | Normal | 150 | 25 | 29.7 |
| V | Sympathectomized | 150 | 25 | 28.2 |
| I | Normal | 125 | 10 | 21.1 |
| I | Sympathectomized | 125 | 10 | 19.3 |

TABLE 3

*Blood lactate of dog I after sympathectomy (room at 14–18°C.)*

| TIME | RATE OF WORK | DURATION OF WORK | BLOOD LACTATE AT END | MAXIMUM HEART RATE |
|---|---|---|---|---|
| | *kgm. m/min.* | *min.* | *mgm. per cent* | |
| Before operation | 125 | 10 | 21.1 | 220 |
| 15 days after | 125 | 10 | 29.2 | 172 |
| 25 days after | 125 | 10 | 15.2 | 205 |
| 35 days after | 125 | 10 | 19.3 | 186 |

were reached. At the end of 4 hours, the normal dogs had regained their resting levels, but the sympathectomized dogs still had high blood lactates.

Part of this difference in reaction may be attributed to removal or denervation of the adrenal medullae. In one dog with an intact sympathetic nervous system, one adrenal removed and the other demedullated, and in another with one adrenal removed and the other denervated, the blood lactate response to adrenalin injection was intermediate between that of sympathectomized dogs and that of normal dogs (fig. 1). The increase in blood lactate after adrenalin injection appears to depend not only on the adrenal medulla but also on the rest of the sympathetic nervous system.

Blood lactate is not necessarily quantitatively related to blood sugar. It is shown in another paper (Brouha, Cannon and Dill, in press) that after adrenalin, blood sugar increases similarly in normal and in sympathectomized dogs, unlike the blood lactate. Hyperglycemia after glucose ingestion alone is not associated with increase in blood lactate. When hyperglycemia was induced by feeding glucose and simultaneously injecting adrenalin, the blood lactate response of both normal and sympathectomized dogs was the same as after adrenalin alone.

In one dog we destroyed all the nerves running along the hepatic artery. After a week, when the dog was again well, we injected adrenalin. The blood lactate response was not greater than before the operation.

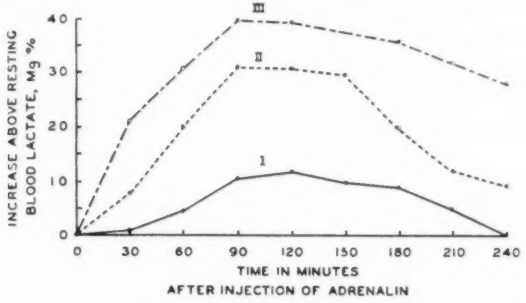



Fig. 1. Blood lactate following subcutaneous injection of 0.1 mgm. adrenalin hydrochloride per kilogram of body weight. Values show increase above resting value in milligrams of lactic acid per 100 cc. blood.

*I.* Normal dogs (average of 12 experiments on 9 dogs).

*II.* Adrenalectomized dogs (average of 3 experiments on 2 dogs).

*III.* Sympathectomized dogs (average of 12 experiments on 8 dogs).

Discussion. The abnormally great accumulation of lactate in the blood of sympathectomized dogs after adrenalin injection might be explained if lactate production were greater in sympathectomized than in normal dogs, if lactate removal were slower, or if both these conditions were present. Lactate is produced principally by the muscles and one might expect that if increased production were responsible for the rise, then muscular exercise of the same intensity for the same time would cause a sympathectomized dog to build up a higher blood lactate than a normal dog. Table 2 shows that this is not true for chronically sympathectomized dogs. Lactate is removed from the blood by excretion through the kidney, by formation of glycogen, and by oxidation. There is no evidence that the renal excretion of lactate is abnormal in the sympathectomized dogs, since their resting blood lactates are normal, and their lactate recovery curves after exercise are normal. We have no evidence concerning tissue oxidation and liver function in sympathectomized dogs. A simple ex-

planation of the blood lactate response of sympathectomized dogs to adrenalin is that chronic absence of adrenin and sympathin in these dogs in some way sensitizes their tissues so that, whatever the mechanism of blood lactate increase after adrenalin, a given dose causes an abnormally large response.

In any case, the behavior of the blood lactate is one of the characteristics in which a chronically sympathectomized dog is abnormal.

## SUMMARY

1. The increases in blood lactate in exercise are the same in chronically sympathectomized as in normal dogs. Whether sympathectomized or not, dogs show no response in respect of blood lactate after ingesting glucose or after insulin has been injected.

2. The increase in blood lactate after injection of a given dose of adrenalin is greater in adrenalectomized than in normal dogs, and is greatest in totally sympathectomized dogs.

## REFERENCES

BROUHA, L., W. B. CANNON AND D. B. DILL. J. Physiol. **87:** 345, 1936.
DILL, D. B., H. T. EDWARDS AND J. H. TALBOTT. J. Physiol. **77:** 49, 1932.
DULIÈRE, W., Z. BACQ AND L. BROUHA. Arch. Int. Physiol. **38:** 164, 1934.
FRIEDEMANN, T. E., M. COTONIO AND P. A. SHAFFER. J. Biol. Chem. **73:** 335, 1927.
SCOTT, A. H. AND B. N. BERG. J. Biol. Chem. **115:** 163, 1936.

# THE EFFECT OF SURGICAL REDUCTION OF THE AMOUNT OF REACTING TISSUE UPON THE QUANTITATIVE EFFECTIVENESS OF TESTOSTERONE PROPIONATE AND ESTRONE[1]

ROY HERTZ[2] AND ROLAND K. MEYER

*From the Zoology Department, University of Wisconsin, Madison*

Received for publication July 7, 1938

Several investigators have described the quantitative features of the biological effect of androgenic materials upon the seminal vesicles and prostate of the gonadectomized rat (1, 2). Their studies have shown that there exists a direct proportionality between the final weight of seminal vesicles or prostate and the dose of androgen administered. It has similarly been shown that small increments in dosage of estrogens are reflected in proportional weight increases in the uteri of immature female rats (3). Moreover, the phenomenon of compensatory hypertrophy which follows unilateral ovariectomy or adrenalectomy (4, 5) suggests that the ratio between the amount of reactive tissue and the quantity of hormone available may determine the degree of response obtained.

From such findings one might conclude that these reactions represent direct and quantitative utilization of the administered or liberated hormone. It occurred to us that if this inference were correct, it should be possible to demonstrate the reciprocal effect of proportionally increasing the biological effectiveness of a given dose of androgen or estrogen by a quantitative reduction of the amount of tissue upon which it must act.

MATERIALS AND METHODS. *Studies with testosterone propionate:* Young male rats of the Sprague-Dawley colony were used. They were 40 to 45 days of age and weighed between 90 and 110 grams. At the beginning of the experiment 70 animals were castrated and at that time 30 of these also had the left seminal vesicle and its associated coagulating gland removed by dissection under the binocular microscope. Within a few hours of the time of operation daily injections of testosterone propionate[3] in 0.1 cc. of olive oil were begun. These were continued for five injections and the animals were killed 24 hours following the last injection. At autopsy, each seminal vesicle and its associated coagulating gland was separately weighed and weight of the prostate was also determined. At

[1] Supported in part by a grant from the Wisconsin Alumni Research Foundation.

[2] Chapple Brothers' Fellow in Endocrinology.

[3] We wish to thank Dr. Erwin Schwenk of the Schering Corporation for the testosterone propionate and the estrone.

this time careful examination under the binocular microscope was made to check up on the completeness of the earlier removal of the seminal vesicles.

The animals were treated in the following manner: 15 were castrated and given a daily dose of 15 gamma testosterone propionate; 15 were castrated and had one seminal vesicle removed and were given a daily dose of 15 gamma testosterone propionate; 15 were castrated and given a daily dose of 30 gamma testosterone propionate; 15 were castrated and had one seminal vesicle removed and were given a daily dose of 30 gamma of testosterone propionate; 10 were castrated and not injected (table 1).

TABLE 1

*Effect of surgical reduction of amount of reacting tissue upon the response to testosterone propionate*

| OPERATIVE TREATMENT | DAILY DOSE OF TESTOSTERONE PROPIONATE | NUMBER OF ANIMALS | RIGHT SEMINAL VESICLE | LEFT SEMINAL VESICLE | PROSTATE (ALL LOBES COMBINED) | VENTRAL LOBES OF PROSTATE | DORSO-LATERAL LOBES OF PROSTATE |
|---|---|---|---|---|---|---|---|
| | *gamma* | | | | | | |
| Castrated; untreated | 0 | 10 | 7.3 ±0.7 | 7.2 ±0.9 | 30.9 ±2.2 | | |
| Castrated and left seminal vesicle removed | 15 | 15 | 15.8 ±1.0* | Removed | 91.6 ±2.4 | | |
| Castrated | 15 | 15 | 14.3 ±0.6* | 13.73 ±0.8* | 93.86 ±4.2 | | |
| Castrated and left seminal vesicle removed | 30 | 15 | 30.0 ±2.6* | Removed | 125.86 ±6.6 | | |
| Castrated | 30 | 15 | 27.0 ±2.0* | 26.6 ±2.0* | 112.13 ±4.0 | | |
| Castrated; left seminal vesicle and ventral lobes of the prostate removed | 30 | 6 | 23.16 ±2.8* | Removed | | Removed | 36.3 ±2.0 |
| Castrated; left seminal vesicle removed; prostate left intact | 30 | 6 | 30.0 ±2.6* | Removed | 105.1 ±8.1 | 71.8 ±6.4 | 33.3 ±1.7 |

* These figures are the actual weight increment of these organs above the control level; absolute weights can be computed by adding these determinations to the mean control weights; all weights are expressed in milligrams.

In a small series of 6 animals, in addition to castration and the removal of one seminal vesicle, the ventral lobes of the prostate were also removed. These animals were injected with a daily dose of 30 gamma of testosterone propionate and at autopsy the weight of the remaining dorso-lateral lobes of the prostate and of the remaining seminal vesicle was determined.[4] The separate weights of the two parts of the prostate in 6 of the preceding animals which had received 30 gamma testosterone propionate daily had been previously determined for comparison (table 1).

[4] The terminology of Callow and Deanesly is employed (6).

*Studies with estrone:* Thirty-seven young female rats of the Sprague-Dawley strain, 22 days old and weighing between 35 and 40 grams, were ovariectomized and the left uterine horn of sixteen of these was also removed under the binocular microscope. A few hours following the operation daily injections of estrone in 0.1 cc. oil were begun and continued for five injections at the dosage indicated in table 2. The animals were killed 24 hours following the last injection. At autopsy each uterine horn was dissected out separately, pressed flat on absorbent paper so as to eliminate its free fluid content, and weighed. The animals were distributed as to operative and hormonal treatment as shown in table 2.

The data from both the testosterone propionate and estrone studies have been subjected to statistical analysis in accordance with Fisher's formula for small numbers (7).

TABLE 2

*Effect of removal of one uterine horn on the degree of uterine response to estrone*

| OPERATIVE TREATMENT | DAILY DOSE OF ESTRONE | NUMBER OF ANIMALS | RIGHT UTERINE HORN | LEFT UTERINE HORN |
|---|---|---|---|---|
| | *gamma* | | | |
| Ovariectomized; untreated | 0 | 7 | 8.42 ±0.6 | 8.57 ±0.4 |
| Ovariectomized; left uterine horn removed | 0.5 | 8 | 41.5 ±2.2* | Removed |
| Ovariectomized; uterus intact | 0.5 | 7 | 38.14 ±3.0* | 38.74 ±2* |
| Ovariectomized; left uterine horn removed | 1 | 8 | 51.75 ±2 | Removed |
| Ovariectomized; uterus intact | 1* | 7 | 57.28 ±2.4* | 54.15 ±1.4* |

* These figures are the actual weight increment of these organs above the control level; absolute weights can be computed by adding these determinations to the mean control weights; all weights are expressed in milligrams

RESULTS AND DISCUSSION. The data obtained from the testosterone studies indicate quite decisively that reduction of the amount of reacting tissue does not quantitatively alter the effect of a given dosage of hormone upon the remaining tissue (table 1). Nevertheless, a 100 per cent increase in dosage practically doubled the weight increment of the reactive organs. This increased response to the higher dosage of hormone is also not affected by experimentally reducing the initial quantity of affected tissue. Thus, administration of 15 gamma of testosterone propionate daily yielded a mean weight increment of 15.8 ±1 mgm. in the right seminal vesicle, when the left had been removed, and a mean weight increment of 14.3 ±0.6 mgm. in the right seminal vesicle and 13.73 ±0.8 mgm. in the left seminal vesicle when both organs remained in situ. Similarly, a daily dose of 30 gamma of testosterone propionate gave a mean weight increment in

the right seminal vesicle of 30.0 ±2.6 mgm. when the left had been removed, and a mean weight increment of 27.0 ±2.0 and 26.6 ±2 mgm. in the right and left seminal vesicles respectively when they were both left intact. The slight differences in the final weight of each seminal vesicle in animals possessing one or two seminal vesicles are not statistically significant.

More drastic reduction of the quantity of sensitive tissue by the removal of practically 70 per cent of the prostate gland and one seminal vesicle did not significantly change the degree of response in either the remaining seminal vesicle or prostatic tissue (table 1). The dorso-lateral lobes of the prostate averaged 33.3 ± 1.7 mgm. after the administration of a daily dose of 30 gamma of testosterone propionate in the presence of the ventral lobes of the prostate, which themselves averaged 71 mgm. in weight. In the absence of the latter, the same dose yielded dorso-lateral prostatic lobes weighing 36.3 ±2 and the remaining single seminal vesicle showed a mean weight increment of 23.16 ±2.8 mgm.

Similar observations on the quantitative action of estrone on the uteri of the ovariectomized immature rat indicate that essentially the same principles apply to the action of the female sex hormone. Under stimulation of a daily dose of 0.5 gamma of estrone the mean weight increment of the right uterine horn was 41.5 ±2.2 mgm. when the left horn had been removed. With the uterus intact this dosage produced a mean weight increment of 38.14 ±3 mgm. and 38.74 ±2 mgm. for the right and left uterine horns respectively. The higher daily dose of 1 gamma of estrone gave comparable results, but at a higher weight level (table 2).

The data, therefore, justify the conclusion that the amount of reacting tissue is not a significant factor in the quantitative response of the seminal vesicles and prostate to testosterone propionate or of the uterus to estrone. This would render improbable the direct quantitative utilization of the hormone in the affected tissues, but suggests that the concentration of the hormone in the blood stream during the effective period may be a significant factor in the determination of the quantitative response to androgens and estrogens. Kochakian (8) has recently shown that prolongation of the interval between injections of certain androgens substantially reduces their efficacy. Such data further support the idea that the level of circulating hormone throughout the test period is a more critical factor in determining the final quantitative response than is the ratio between the dosage and the amount of reactive tissue.

## SUMMARY

The effect of surgically reducing the amount of reactive tissue upon the quantitative response to testosterone propionate and estrone was tested in the rat. Seventy-six young male rats, 90 to 110 grams in weight,

were all gonadectomized; thirty of them also had one seminal vesicle and its associated coagulating gland removed, and six had the ventral lobes of the prostate and one seminal vesicle removed. Thirty animals received 15 gamma of testosterone propionate daily for 5 days, thirty-six animals received 30 gamma of testosterone propionate daily for 5 days, and ten were not injected. Similarly, 37 female rats, 22 days of age, were ovariectomized and the left uterine horn was also removed from 16 of them. Fifteen of them received 0.5 gamma of estrone daily for 5 days, 15 received 1 gamma of estrone daily for 5 days, and seven were not injected. Autopsy weights of seminal vesicles, prostates, and uterine horns show that the surgical removal of considerable portions of the reactive tissue does not alter the quantitative response of the remaining tissue to either testosterone propionate or estrone. The data suggest that the concentration of hormone in the blood stream during the experimental period is a more significant factor in the determination of the quantitative response than is the ratio of hormone dosage to the amount of reactive tissue.

## REFERENCES

(1) Tschopp, E. Arch. intern. de pharmacodyn. et de therap. **52:** 381, 1936.
(2) Deanesly, R. and A. S. Parkes. Biochem. J. **30:** 291, 1936.
(3) Severinghaus, E. L., C. Heller and H. Lauson. Proc. Am. Soc. Biol. Chemists, 1938, p. 107.
(4) Hatai, S. J. Exper. Zool. **15:** 297, 1913.
(5) MacKay, E. M. and L. L. MacKay. J. Exper. Med. **43:** 395, 1926.
(6) Callow, R. K. and R. Deanesly. Biochem. J. **29:** 1424, 1935.
(7) Davenport, C. B. and M. P. Ekas. Statistical methods in biology. John Wiley & Sons, New York, 1936.
(8) Kochakian, C. D. Endocrinology **22:** 181, 1938.

# THE THERMAL TOLERANCE OF NORMAL RESTING DOGS AS MEASURED BY CHANGES OF THE ACID BASE EQUILIBRIUM AND THE DILUTION-CONCENTRATION EFFECT OF PLASMA

ALLAN HEMINGWAY[1] AND HENRY G. BARBOUR

*From the Laboratory of Pharmacology and Toxicology, Yale University School of Medicine, New Haven, Connecticut*

Received for publication July 11, 1938

Animals which are deficient in sweat glands rely upon the process of thermal tachypnea (panting) as a means of evaporating water for purposes of cooling. As a result of excessive over-ventilation due to prolonged exposure to a warm environment there is an "auspumpung" or blowing off of carbon dioxide, as described by Rice and Steinaus (1), Flinn (2) and Anrep and Hammouda (3). This results, under extreme conditions, in a respiratory alkalosis characterized by a low blood bicarbonate and a high plasma pH value. Since the respiratory alkalosis is one of the first signs of a failure to cope with overheating, it can be used in studies of heat tolerance to indicate the physiological limit beyond which the mechanism for heat regulation shows impairment.

Hemingway and McClendon (4) have shown that by using diathermy and measuring high frequency voltage and current it is possible to measure in calories the amount of heat production in the tissues by the diathermy current. A machine for the generation of diathermy current and the measurement of calorie output has been described by Hemingway and Witts (5). Using diathermy it is possible to produce in the tissues of the animal any desired amount of heat at any desired rate. The heat so produced must be eliminated together with the usual metabolic heat. In this way it is possible to measure the amount of heat which an animal must eliminate to maintain a normal temperature, and the heat tolerance of an animal can be measured in quantitative units.

Trained dogs have been given measured dosages of diathermy heat, and blood samples have been drawn at intervals for measurement of plasma pH and carbon dioxide content. Hematocrit determinations were also made which show the dilution-concentration effect described by Barbour and Tolstoi (6), Barbour, Dawson and Neuwirth (7) and Lozinsky (8).

[1] Sterling Fellow, Yale University. On leave of absence from the University of Minnesota 1936-37.

METHODS AND PROCEDURE. The dogs were trained to lie quietly in a respiration plethysmograph and on a table for 5 hours. No anesthetics were used except 0.5 cc. of 5 per cent procaine at the site of arterial puncture. The dogs were heated by tin foil electrodes, one placed on the upper surface and the other beneath each animal. Two series of experiments were made in which environmental conditions and heat dosages were the same. In one series arterial blood was drawn for analysis from the heated animals lying on a table. In the other series the animals were sealed at the neck in a body plethysmograph and measurements were made of minute volume of respiration. In all experiments the room temperature was 30–31°C. with the relative humidity 40–60 per cent.

TABLE 1

*Blood analyses*

| HEAT DOSAGE | 1.0 B.M.R. | | | | 2.0 B.M.R. | | | | 3.0 B.M.R. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH | $(CO_2)$ | Hematocrit | $pCO_2$ | pH | $(CO_2)$ | Hematocrit | $pCO_2$ | pH | $(CO_2)$ | Hematocrit | $pCO_2$ |
| Dog A | | | | | | | | | | | | |
| Basal | 7.37 | 23.3 | 0.432 | 41.5 | 7.35 | 24.3 | 0.457 | 45.0 | 7.35 | 24.3 | 0.522 | 45.0 |
| After 1 hour | 7.40 | 24.2 | 0.411 | 40.5 | 7.35 | 23.8 | 0.430 | 44.0 | 7.40 | 23.3 | 0.519 | 39.0 |
| After 2 hours | 7.37 | 23.5 | 0.389 | 41.5 | 7.34 | 24.3 | 0.510 | 46.0 | 7.39 | 24.0 | 0.512 | 40.5 |
| After 3 hours | 7.46 | 23.1 | 0.415 | 33.2 | 7.35 | 24.4 | 0.529 | 45.0 | 7.39 | 24.1 | 0.532 | 40.5 |
| Dog B: | | | | | | | | | | | | |
| Basal | 7.39 | 22.6 | 0.450 | 38.3 | 7.48 | 24.3 | 0.454 | 33.5 | 7.41 | 25.5 | 0.497 | 41.5 |
| 1 hour | 7.40 | 21.8 | 0.446 | 36.0 | 7.45 | 23.2 | 0.427 | 34.5 | 7.42 | 23.5 | 0.533 | 37.5 |
| 2 hours | 7.43 | 22.4 | 0.426 | 35.0 | 7.48 | 23.9 | 0.417 | 34.0 | 7.46 | 21.9 | 0.543 | 32.0 |
| 3 hours | 7.39 | 22.3 | 0.386 | 37.5 | 7.44 | 23.5 | 0.445 | 33.5 | 7.50 | 21.0 | 0.560 | 31.0 |
| Dog C: | | | | | | | | | | | | |
| Basal | 7.38 | 20.6 | 0.491 | 35.8 | 7.25 | 20.7 | 0.482 | 48.0 | | | | |
| 1 hour | 7.27 | 18.8 | 0.475 | 42.5 | 7.33 | 18.1 | 0.457 | 35.0 | | | | |
| 2 hours | 7.35 | 18.7 | 0.441 | 34.8 | 7.34 | 17.4 | 0.466 | 33.5 | | | | |
| 3 hours | 7.38 | 18.8 | 0.448 | 33.2 | 7.40 | 14.8 | 0.445 | 30.5 | | | | |

In the first series the dogs rested for 2 hours with two "basal" samples of blood being drawn near the end of this period and the rectal temperature measured. The diathermy current was then started and continued for 3 hours. At the end of each hour a sample of blood was drawn without the current being interrupted. The diathermy current was adjusted to give heat dosages which were integral multiples of the b.m.r. as computed from the data of Kitchen (9). Thus with a b.m.r. dosage of 2.0 the animal was required to dissipate an amount of heat equal to twice the b.m.r. as well as his own metabolic heat, the total heat elimination being slightly greater than three times the b.m.r. Two dogs were given heat treatments with diathermy of 1.0, 2.0 and 3.0 times the b.m.r. while the third dog was given only 1.0 and 2.0 times the b.m.r., as shown in table 1. At least 3 days separated successive experiments on a single dog.

About 8 ml. of arterial blood were drawn under oil into a calibrated centrifuge tube containing 0.5 ml. of iso-osmotic sodium oxalate. The blood was immediately chilled and centrifuged in the cold, this procedure, according to Yoshimura (10) being necessary to prevent the acid change described by Havard and Kerridge (11). The pH was measured by the glass electrode method described by Du Bois (12). The cell and plasma volumes were determined and the plasma carbon dioxide content measured by the Van Slyke manometric procedure. The results are given in table 1.

On another day with similar experimental conditions the dogs were sealed into a body plethysmograph. After a two hour rest they were given a diathermy treatment of the same value as those of the first series. The plethysmograph was maintained at room temperature by a cooling system consisting of a pump and cooling coils. A stream of air was withdrawn from the plethysmograph, cooled and directed back into the closed box. The air flow through the cooling coils was adjusted to maintain the temperature of 30–31°C. within the plethysmograph. The plethysmograph (13) was designed to record true respiratory volumes at the high respiratory rates such as occur in thermal tachypnea of dogs where respiratory rates exceed 300 per minute.

Results. The plasma pH values, the $CO_2$ content of plasma in millimols per liter and the hematocrit values of blood samples drawn before, and at hourly intervals during, the three hour diathermy treatment at the respective heating rates are given in table 1.

The alveolar $CO_2$ tension ($pCO_2$), in millimeters Hg, has been computed from the triaxial coördinate graph of the Henderson-Hasselbalch equation given by Hastings and Steinhaus (14). The average hourly hematocrit values for the three dogs heated at a rate of 3 b.m.r. and three dogs heated at rates of 2.0 and 1.0 b.m.r. are given in figure 1.

The pH of normal dog plasma obtained from dogs under basal conditions varied from 7.25 to 7.48 at 38 degrees. This blood was drawn from trained dogs after the rest preceding the heat treatment. These measurements were made with the glass electrode in which precautions were taken to eliminate the "first acid shift", and the values fall within the ranges described by others. Stadie, Austen and Robinson's (15) values at 38 degrees range from 7.08 to 7.58 while similar values of Cullen, Keeler and Robinson (16) are 7.102 to 7.350 at 38 degrees.

With heat dosages of 1.0 b.m.r. for a three hour diathermy treatment for dogs A and B there were irregular fluctuations in the acid base balance but no significant changes. Dog C after the first hour showed a definite tendency toward respiratory alkalosis, as indicated by an increasing plasma pH, reduced $CO_2$ content and $pCO_2$. With heat dosages of 2.0 b.m.r. there were no significant changes of the acid base balance of dogs A and B while C showed a definite respiratory alkalosis as indicated by a progressive

rise of pH and fall of $CO_2$ content and alveolar $CO_2$ tension. With a heating rate of 3.0 b.m.r., dog B had a definite respiratory alkalosis while dog A was well able to tolerate this heat treatment as judged by the state of the acid base equilibrium. Dog C which had a mild respiratory alkalosis with a heating rate of 1.0 b.m.r. and a severe alkalosis with 2.0 b.m.r. was an obese terrier of 15.7 kilos and exhibited signs of discomfort and violent panting with a heat dosage of 2.0 b.m.r. As indicated by this procedure the tolerance limit was reached and a heating rate of 3.0 b.m.r. was discontinued since skin burns from the high current density and the subcutaneous fat resulted. Dog A, 18.0 kilos, was a young, thin,

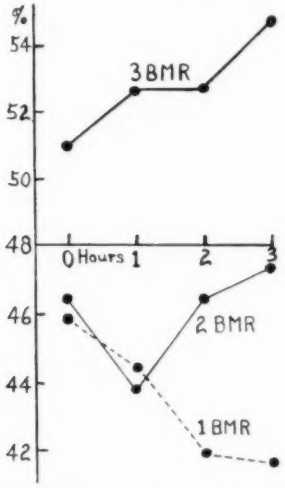



Fig. 1. The average changes in the cell volume of blood during an 8 hour diathermy treatment with heating rates as given. With a 1.0 b.m.r. heat dosage there is only dilution, with 2.0 b.m.r. an initial dilution is followed by concentration and with 3 b.m.r. only concentration occurs. Ordinates: hematocrit readings. Abscissae: time.

long legged, long nosed pointer with large ears and tolerated heating rates of 3.0 b.m.r. without any symptoms of respiratory alkalosis. Dog B, 19.0 kilos, was an older dog, intermediate in nutritive condition and was able to tolerate heating rates of 1.0 and 2.0 b.m.r. but exhibited respiratory alkalosis with rates of 3.0 b.m.r.

The dilution of the blood with mild heating and the concentration with more intense heating, as observed by Barbour and co-workers, is shown in figure 1. A mild heat treatment of a rate equal to 1.0 b.m.r. produced only a continued dilution. A heating rate of 2.0 b.m.r. produced dilution followed by concentration while a rate of 3.0 b.m.r. produced only con-

centration. With this highest heating rate it is possible that a transient dilution occurred during the first hour.

The respiratory data are given in table 2. When the diathermy current is turned on there is a brief latent period designated as period 1 during which there is no increase in ventilation rate. Following this period there is a progressive rise in ventilation rate until a maximum ventilation is reached, which usually occurs shortly after panting commences. The period of increasing ventilation rate is period 2. When the maximum has been reached the ventilation continues at a high value for the remainder of the treatment. This is period 3. There is considerable fluctuation during period 3 and the average value for the ventilation rate during this period is given in the table.

TABLE 2

*Respiratory data*

| DOG | HEAT DOSAGE B.M.R. | DURATION OF PERIOD | | | BASAL VENT. RATE | AVERAGE VENT. RATE PERIOD 3 | BASAL TEMP., RECTAL | FINAL PANTING TEMP., RECTAL |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | | |
| | | *min.* | *min.* | *min.* | *l/min.* | | | |
| A | 1 | 0 | 35 | 145 | 5.8 | 12.7 | 38.2 | 38.5 |
| A | 2 | 0 | 60 | 120 | 2.2 | 17.8 | 38.0 | 39.0 |
| A | 3 | 0 | 25 | 155 | 3.0 | 15.6 | 38.2 | 38.5 |
| B | 1 | 15 | 15 | 150 | 7.8 | 22.7 | 37.9 | 38.5 |
| B | 2 | 30 | 60 | 90 | 2.6 | 22.9 | 37.6 | 38.8 |
| B | 3 | 15 | 90 | 75 | 3.4 | 16.9 | 38.3 | 38.7 |
| C | 1 | 15 | 15 | 150 | 1.4 | 16.6 | 37.7 | 38.4 |
| C | 2 | 0 | 10 | 170 | 1.9 | 14.9 | 38.0 | 38.7 |

Discussion. The respiration of heat treated dogs is subjected to two influences, namely, the tendency to increase ventilation for cooling purposes and the tendency to reduce ventilation if carbon dioxide is lost. It is clearly evident that the body temperature regulating process takes precedence over the $CO_2$ conserving mechanism and that an animal may lose $CO_2$ while preventing overheating. The sweat gland mechanism of man does not have this disadvantage.

An animal can tolerate mild heat without losing $CO_2$. With a heat treatment of 1.0 b.m.r. there is a considerable hyper-ventilation but no loss of $CO_2$. With higher heat dosages symptoms of respiratory alkalosis develop, especially in dogs which are obese and which have a poor regulating mechanism.

A respiratory alkalosis can only be diagnosed if both pH and $CO_2$ content are simultaneously determined. Unfortunately in much of the older work, i.e., Flinn (2) and Anrep and Hammouda (3) only $CO_2$ content was determined. Anrep and Hammouda (3) have made the interesting

observation that lactic acid increases during the exposure of anesthetized dogs to heat. If this were appreciable it would lead to a *decrease* of pH with a decrease of $CO_2$. Haggard (16) in man finds no change in $CO_2$ capacity but a decrease in $CO_2$ content indicating that an acidosis due to increased fixed acids is inappreciable.

Lozinsky (8) has measured the changes in blood solids of dogs when exposed to warm environments. The changes follow a general course of dilution followed by concentration. The dilution occurs during mild panting and is a normal response to heat. The concentration which follows is due to loss of water and occurs after more prolonged and severe heating. Our results agree with those of Lozinsky. During the early stages of a mild heat treatment there is usually dilution as indicated by the hematocrit. In the last hour of a mild treatment, or earlier with higher dosages, the concentration effect is noted. Since these dogs had all been carefully trained, and struggling and excitement were rarely found, an epinephrine effect of splenic contraction and increased hematocrit due to excitement is unlikely.

The respiratory data of table 2 show that during thermal tachypnea of dogs the ventilation rate increases 2 to 10 times the basal values. This hyperventilation undoubtedly results in loss of $CO_2$. There is apparently no definite relation between the magnitude of the hyperventilation and the heating rate. In all cases the hyperventilation increased to a maximum which remained independent of heat dosage. The differences in response in periods 1 and 2 may be due to the fact that although the panting rectal temperatures were maintained within a narrow range between 38.85 and 39.05 degrees, the basal temperatures differed widely, i.e., 37.6 to 38.3. The amount of "storage heat" would therefore vary with the basal temperature. Since it was necessary to conduct one experiment for the collection of blood samples and another for the measurement of ventilation rate, the day to day variability would be a factor in these experiments.

## SUMMARY

Ventilation rate, arterial plasma pH and $CO_2$ content have been determined for three trained dogs given measured heat dosages by diathermy equal to 1.0, 2.0 and 3.0 times their b.m.r. for three hours, at a room temperature of 30–31° and a relative humidity of 50± 5 per cent. One dog, the most obese of the three, showed the lowest heat tolerance and developed a respiratory alkalosis, as indicated by reduced $CO_2$ content and increased pH, with a heat dosage of 2.0 b.m.r. With a heat dosage of 3.0 b.m.r., the second dog developed respiratory alkalosis while the third showed no signs of this condition, being particularly tolerant to heat.

Heating with 1 b.m.r. diluted the blood, 2 b.m.r. caused dilution followed by concentration, while in two dogs 3 b.m.r. caused only blood concentra-

tion as measured hourly for three hours by the hematocrit method. As a result of the heat treatment the ventilation rate increased two- to ten-fold (average five-fold).

We wish to acknowledge the aid and coöperation of Dr. D. I. Hitchcock and Mr. D. Du Bois in the glass electrode measurements of plasma pH.

REFERENCES

(1) Rice, H. A. and A. H. Steinaus. This Journal **96:** 529, 1931.
(2) Flinn, F. B. Pub. Health Repts. **40:** 868, 1925.
(3) Anrep, G. V. and M. Hammouda. J. Physiol. **77:** 16, 1932.
(4) Hemingway, A. and J. F. McClendon. Physics **4:** 351, 1933.
(5) Hemingway, A. and G. E. Witts. Physics **7:** 299, 1936.
(6) Barbour, H. G. and E. Tolstoi. This Journal **67:** 378, 1923.
(7) Barbour, H. G., M. H. Dawson and I. Neuwirth. This Journal **74:** 204, 1925.
(8) Lozinsky, E. This Journal **67:** 388, 1923.
(9) Kitchen, H. D. This Journal **67:** 487, 1923.
(10) Yoshimura, H. J. Biochem. (Jap.) **22:** 279, 1935.
(11) Havard, R. E. and P. T. Kerridge. Biochem. J. **23:** 600, 1929.
(12) Du Bois, D. J. Biol. Chem. **88:** 729, 1930.
(13) Hemingway, A. Science **87:** 47, 1938.
(14) Hastings, A. B. and A. H. Steinaus. This Journal **96:** 538, 1931.
(15) Stadie, W. C., J. H. Austin and H. W. Robinson. J. Biol. Chem. **66:** 901, 1925.
(16) Cullen, G. E., H. R. Keeler and H. W. Robinson. J. Biol. Chem. **66:** 301, 1925.
(17) Haggard, H. W. J. Biol. Chem. **44:** 131, 1920.

# CHEMICAL MEDIATORS IN THE AQUEOUS HUMOR

J. V. LUCO[1] AND K. LISSÁK[2]

*From the Department of Physiology in the Harvard Medical School*

Received for publication June 28, 1938

Engelhart (1931) found that either direct or reflex stimulation of the parasympathetic nerves of the eserinized eye of the rabbit releases an acetylcholine-like substance into the aqueous humor (a.h.). This substance is rendered ineffective by atropine when tested on an isolated toad heart. Bacq (1931, 1933) reported that in the a.h. of the rabbit and dog a sympathomimetic substance is present when the cervical sympathetic is stimulated; this substance was tested on the toad heart and on the cat's pilomotor muscles.

The purpose of the present study was to ascertain whether the amount of sympathin released by electrical stimulation of a denervated superior cervical ganglion differs from the amount released by stimulation of the normally innervated ganglion. In order to interpret the results obtained other experiments on the release of the chemical mediators in the a.h. were performed.

METHOD. Rabbits and cats were used, anesthetized with dial (Ciba, 0.6 and 0.75 cc. per kilogram intraperitoneally, respectively). A cannula was inserted into the trachea for artificial respiration, when necessary. The stimulus employed was a faradic current for 10 minutes from a Harvard inductorium, with five volts in the primary circuit. The stimulating electrodes were shielded silver wires. Maximal stimulation was always used.

The a.h. was slowly collected into a syringe through a small needle. In some animals the cervical sympathetic chain was cut under aseptic conditions and the postganglionic fibers stimulated from 6 to 20 days later. The drugs which were used were injected into the femoral vein. In some experiments physostigmine (1:100) was dropped on the eyes. The parasympathetic nerves were reflexly stimulated by exposure of the eye to a strong electric lamp for 10 minutes. The lamp was always placed at a fixed distance from the cornea.

The a.h. was tested, not later than 15 minutes after being collected, on isolated hearts of spring frogs, perfused according to the method of

[1] John Simon Guggenheim Fellow from Chile.

[2] Rockefeller Fellow from Hungary.

Straub. The small amount of a.h. (0.2 cc.) obtained from rabbits allows only one titration on the frog heart. In each experiment the a.h. collected from the cat (1 cc.) was tested three times.

When used for testing for sympathin the heart was washed several times, to render it hypodynamic, and one part of a.h. was diluted in three parts of Ringer's solution. The a.h. of the rabbit contains proteins which can affect the heart. Their action was differentiated from the sympathin reaction, because the latter, like the adrenaline effect, appears in a few seconds and disappears rapidly after washing, whereas the protein effect appears later and takes a long time to disappear after washing.

As an indicator of acetylcholine a normal heart was used, previously perfused with frog Ringer's solution containing physostigmine (1:100,000). In the test 1 part of a.h. was diluted in 2 parts of Ringer. Atropine (2 or 3 drops of a 1:10,000 solution) was added later to test whether or not the inhibition was due to acetylcholine.

RESULTS. A. *Sympathin in the aqueous humor of the rabbit.* Twenty-seven animals were used. In some instances the postganglionic fibers of the cervical sympathetic were stimulated; in others no stimulation was used. In 12 rabbits the preganglionic trunk was cut on one side 6 to 20 days before. In the animals in which the sympathetic was not cut previously the preganglionic fibers were acutely severed.

The a.h. collected from normal rabbits without stimulation has in many instances a sympathomimetic action on the frog heart. This action varies in intensity. After stimulation of the sympathetic on one side, the corresponding a.h. had, in 3 out of 8 experiments, a sympathomimetic action; in these 3 cases the normal side had no such action. In the other 5 experiments the reactions from the stimulated side and the non-stimulated side were equal (fig. 1). Conversely, although stimulation of the two cervical sympathetics led sometimes to equal activity of the a.h. from the two sides, in other cases the effects were different. These discrepancies probably reflect differences in the sympathomimetic action of the normal a.h. The results observed on normal rabbits are summarized in table 1.

To learn whether the sympathomimetic action of the normal a.h. is related merely to sympathetic innervation (without special stimulation), the a.h. of 2 rabbits in which the preganglionic fibers of one side were chronically cut, and of one rabbit with one superior cervical sympathetic ganglion removed for 10 days, was collected and tested on the frog heart. The sympathomimetic action of the a.h. of the two sides was the same (fig. 2).

The results in operated rabbits were similar to those found in the normal animals. The a.h. collected after the stimulation of both sides had in some experiments the same sympathomimetic action; but in others a

different one. It was greater sometimes from the denervated side and sometimes from the normal side. Table 2 shows a summary of the results obtained on operated rabbits.

B. *Sympathin in the aqueous humor of the cat.* Thirty-one cats were used; 19 were normal and 12 had had the preganglionic cervical sympathetic trunk cut on one side from 6 to 20 days previously. In the normal animals and on the normal side of the operated cats the preganglionic fibers were acutely severed.

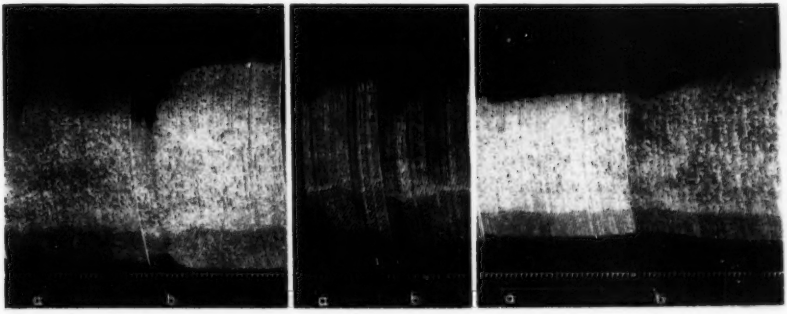



Fig. 1 Fig. 2 Fig. 3

Fig. 1. Hypodynamic frog heart. In this and succeeding records the time signal marks 5-second intervals. At *a*, aqueous humor (a.h.) of the non-stimulated side (rabbit); at *b*, a.h. of the stimulated side.

Fig. 2. Hypodynamic frog heart. At *a*, a.h. of the operated side of a rabbit (supr. cerv. symp. ganglion removed 10 days previously); at *b*, a.h. of the normal side.

Fig. 3. Hypodynamic frog heart. At *a*, a.h. of stimulated side of a cat before cocaine; at *b*, a.h. of the other side stimulated after cocaine.

TABLE 1

*Normal rabbits*

| | NEITHER SIDE STIMULATED | ONE SIDE STIMULATED | BOTH SIDES STIMULATED |
|---|---|---|---|
| Equal sympathomimetic action from both sides | 1 | 5 | 3 |
| Sympathomimetic action only from stimulated side | | 3 | |
| Different action from the two sides | | | 3 |

In the cat, unlike the rabbit, the results were more consistent, probably because the a.h. of normal cats has no sympathomimetic action. Stimulation of the cervical sympathetic, as Bacq (1931, 1933) reported, does not release detectable amounts of sympathin in the a.h. After injection of cocaine (8 mgm. per kgm.), however, stimulation of the postganglionic fibers releases sympathin (fig. 3). When the two sides are equally stimulated in normal cats, after cocaine, the amount of sympathin is the same from the two sides (fig. 4).

When the postganglionic fibers were stimulated, without cocaine, in 4 cats with previous preganglionic denervation sympathin did not appear in the a.h.

TABLE 2

*Operated rabbits*

| | PREGANGLIONIC DENERVATION ON ONE SIDE | | SUPERIOR CERVICAL SYMPATHETIC GANGLION REMOVED ON ONE SIDE |
|---|---|---|---|
| | Neither side stimulated | Both sides stimulated | Neither side stimulated |
| Equal sympathomimetic action from the two sides | 2 | 2 | 1 |
| Greater sympathomimetic action from normal side | | 4 | |
| Greater sympathomimetic action from operated side | | 2 | |
| Sympathomimetic action only from normal side | | 1 | |

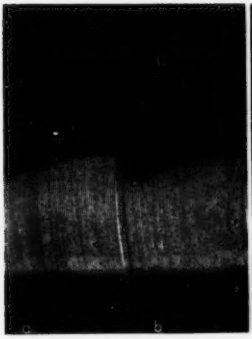


Fig. 4

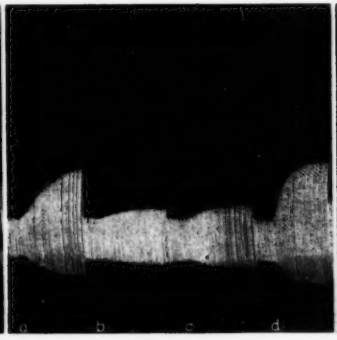


Fig. 5

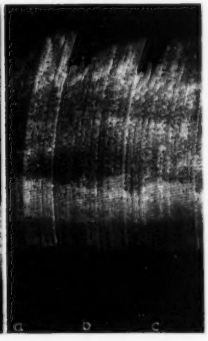


Fig. 6

Fig. 4. Hypodynamic frog heart. At *a* and at *b*, a.h. of each side of a cat stimulated after cocaine.

Fig. 5. Hypodynamic frog heart. At *a*, a.h. of the preganglionically denervated side of a cat stimulated, after cocaine; at *b*, a.h. of the normal side stimulated after cocaine; at *c*, adrenalin (1:1,000,000,000); at *d*, adrenalin (1:500,000,000).

Fig. 6. Hypodynamic frog heart. At *a*, adrenalin (1:1,000,000,000); at *b*, a.h. of a stimulated side of a cocainized cat before 933F; at *c*, after 933F.

In 7 of 8 experiments performed on animals with one side preganglionically denervated the amount of sympathin in the a.h. of the denervated side, stimulated after an injection of cocaine, was greater than that of the normal side (fig. 5). In the other experiment similar concentrations of sympathin occurred on the two sides.

In 3 normal cats cocaine was injected and the postganglionic fibers were stimulated on one side. The sympathin released in the a.h. was tested on the frog heart. Thereafter, 933F (3 mgm. per kgm.) was injected and the postganglionic fibers of the other side were stimulated. The amount of sympathin released after 933F was the same as that which had been detected before the injection of the drug (fig. 6).

C. *Acetylcholine in the aqueous humor of rabbits.* Fifteen animals were used: 10 normal and 5 in which the superior cervical sympathetic ganglion had been removed 7 to 10 days previously.

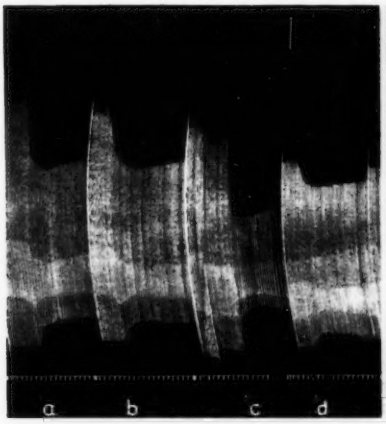



Fig. 7

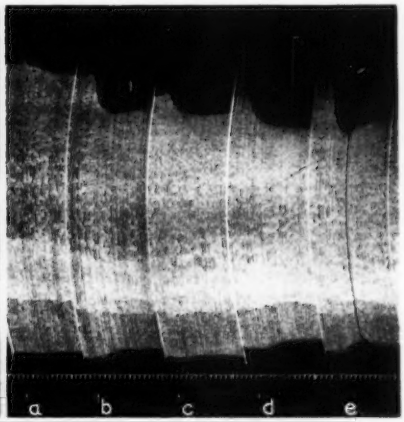



Fig. 8

Fig. 7. Normal frog heart. At *a*, a.h. of the operated side of a rabbit (supr. cerv. symp. ganglion removed 12 days previously) exposed to light; at *b*, the normal side exposed to light; at *c*, acetylcholine (1:100,000,000); at *d*, acetylcholine (1:1,000,000,000).

Fig. 8. Normal frog heart. At *a*, acetylcholine (1:1,000,000,000); at *b*, acetylcholine (1:100,000,000); at *c* and at *d*, the a.h. of each side of a cat reflexly stimulated with a light; at *e*, acetylcholine (1:100,000,000).

In the normal rabbits, Engelhart's observation was confirmed, i.e., in a previously eserinized eye reflex stimulation of the parasympathetic nerves produces acetylcholine in the a.h., while the eserinized but not stimulated side has no acetylcholine. When both sides were equally stimulated equal amounts of acetylcholine were found.

In the operated animals the amount of acetylcholine in the a.h. of the two sides after stimulation of the eserinized eyes was the same (fig. 7). It may be concluded that sympathetic denervation does not modify the amount of acetylcholine released upon stimulation of the IIIrd nerve.

D. *Acetylcholine in the aqueous humor of cats.* Six animals were used;

3 normal and 3 in which the superior cervical sympathetic ganglion of one side had been removed 7 to 10 days before. In all the experiments both eyes were eserinized 2 to 3 hours before the test, and the parasympathetic nerves were reflexly stimulated.

In the 3 normal cats one eye was stimulated before cocaine (8 mgm. per kgm.) and the other after the injection of the drug. In these cases equal small amounts of acetylcholine appeared in the a.h. of the two sides.

In two of the operated cats no acetylcholine was found in either side after bilateral reflex. In the third cat equal small amounts were detected in the two sides after such stimulation (fig. 8).

Discussion. The results reported in section A show clearly that the normal a.h. of the rabbit very often increases the inotropism of the hypodynamic frog heart (fig. 1). The more sensitive the heart to adrenaline, the stronger is this action. Bacq (1931; 1933) reported a similar effect on the toad heart. This sympathomimetic action of the a.h. is not due to sympathetic stimulation from ether anesthesia, as Bacq suggests, because under dial, which does not cause sympathetic stimulation, it is also present; and also because it still occurs 10 days after cutting the cervical sympathetic (fig. 2). The factor responsible for this sympathomimetic action of the normal a.h. is still unknown; it may be chemically related to adrenaline, since in 4 out of 10 experiments Bacq (1933) found that the Bayer reaction was positive on both the normal and the stimulated side.

Bacq (1933) expressed the opinion that the frog heart is not as good an indicator for sympathin as the toad heart, because the fluid from unstimulated eyes is more apt to yield a positive inotropic effect on the former than on the latter. It is probable that this difference may be attributed to the greater sensitivity of the hypodynamic frog heart to many agents, adrenaline and sympathin included.

The present observations lead to the conclusion that the rabbit is an unfavorable animal in which to study the appearance of sympathin in the a.h. Indeed, with the evidence available (Bacq's and the present) it is still a question whether any sympathin diffuses into the a.h. of the rabbit when the sympathetic supply to the iris is activated.

The experiments reported in section B confirm Bacq's (1931, 1933) observation that in normal cats the a.h. has no detectable sympathomimetic substances and that cervical sympathetic stimulation does not release measurable concentrations of sympathin in the a.h. A release of sympathin occurs, however, when cocaine has been injected before stimulation (fig. 3).

The mechanism of this action of cocaine is unknown. It might be explained as due to a larger amount of sympathin released per nerve impulse; but this suggestion does not account for the increased responses

to adrenalin after injections of the drug. Cocaine might increase cellular permeability (Rosenblueth, 1932); but such a change in the permeability of the cells is probably not an important factor for the release of sympathin in the a.h., because after preganglionic denervation—supposed also to increase cellular permeability—sympathin is not present in the a.h. after stimulation (section B). Another possibility is that cocaine may inhibit the oxidation of sympathin. This suggestion would account for the longer responses to sympathetic stimulation after cocaine (Rosenblueth and Cannon, 1931), and it seems to be the most reasonable explanation for the release of sympathin in the a.h. of cocainized cats after sympathetic stimulation. Such an explanation is supported by the observation that cocaine *in vitro* inhibits adrenaline oxidation (Bayer, 1937, 1938) and is in accord with Bonhomme's (1936) observation that the anti-oxygen power of the a.h. of the cat is weaker than that of other animals.

The arguments used above in discussing the effect of cocaine may be used to explain the larger amount of sympathin found in the a.h. of cocainized cats in the preganglionically denervated side as compared with the normal side (fig. 5). As pointed out before, an increase of cellular permeability would not seem to be an important factor. A decrease of the rate of sympathin oxidation probably is not the condition responsible, since cocainization would inhibit oxidation on both sides. It is possible to explain the phenomenon by assuming that the amount of sympathin released per nerve impulse on the preganglionically denervated side is larger than produced normally.

The lack of influence of sympathetic denervation on the release of acetylcholine (sections C and D, fig. 7) may be explained by assuming that the cells modified by such denervation differ from the elements which play a rôle in the liberation of acetylcholine.

Engelhart (1931) failed to detect any acetylcholine in the a.h. of the cat. In 4 out of the present 6 experiments (section D, fig. 8) slight but clearly detectable amounts were found in experimental conditions similar to those described by Engelhart. This difference of results may be due to the fact that Engelhart used cats under urethane anesthesia and the toad heart as a test organ, while we used dial as anesthetic and the frog heart as indicator.

## SUMMARY

The liberation of the chemical mediators in the aqueous humor, after stimulation of the sympathetic and parasympathetic nerves of the eyes, has been studied in 79 experiments performed on rabbits and cats.

The results obtained on rabbits were so inconsistent that it is impossible to draw any conclusion from them (figs. 1 and 2).

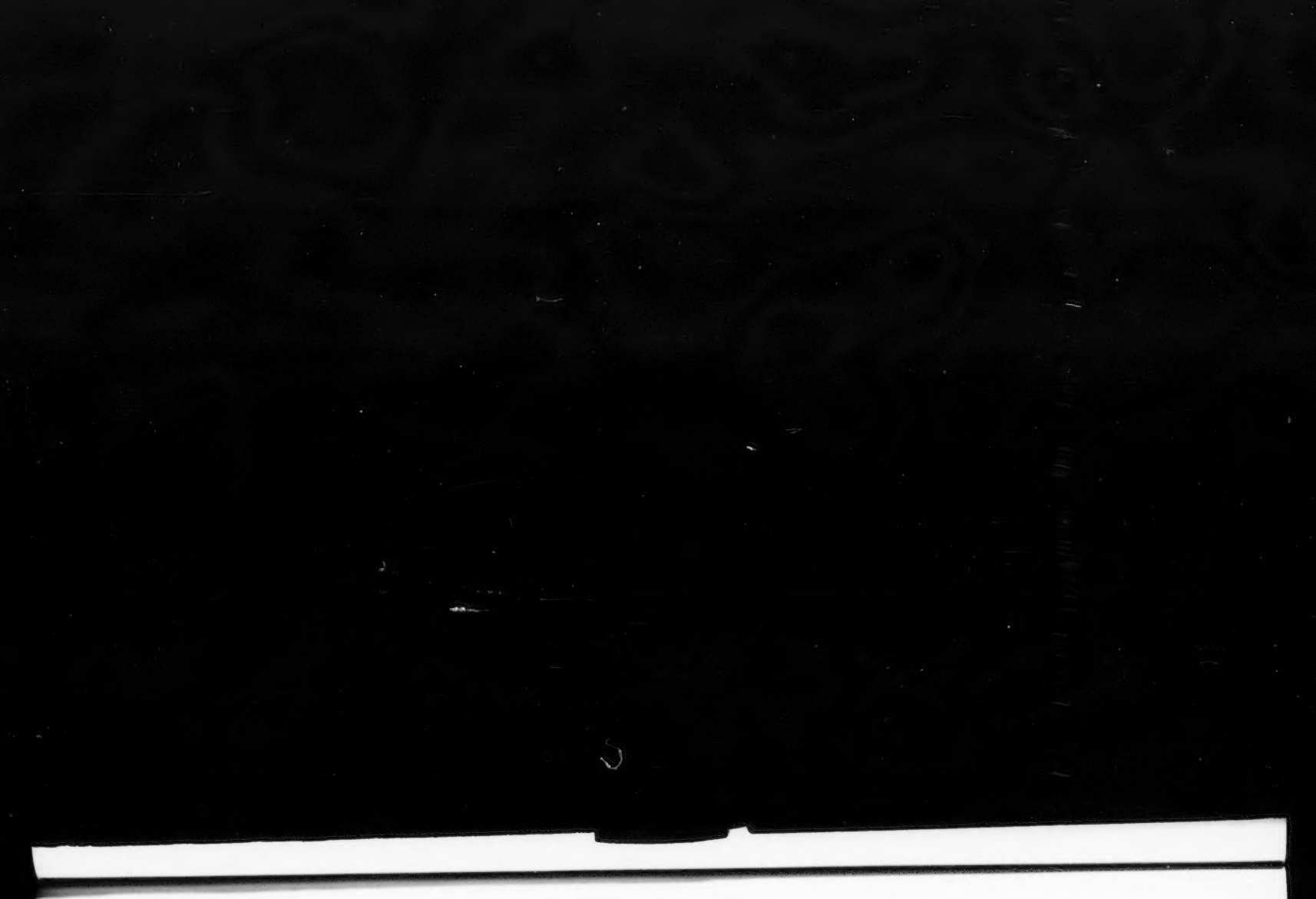
On normal and on operated cats (preganglionic cervical sympathetic

denervation on one side) sympathetic stimulation does not release measurable concentrations of sympathin; but it is demonstrably present if the sympathetic is stimulated after the animal has been cocainized (figs. 3 and 4).

After cocaine the operated side releases a larger amount of sympathin than the normal side under the same postganglionic stimulation (fig. 5).

Engelhart's observation on rabbits was confirmed (fig. 7). On cats similar results were obtained, i.e., reflex stimulation of the parasympathetic nerves of the eye released acetylcholine in the aqueous humor (fig. 8).

It is a pleasure to thank Dr. W. B. Cannon and Dr. A. Rosenblueth for their valuable criticisms during the course of this work.

## REFERENCES

Bacq, Z. M. Compt. Rend. Soc. Biol. **101:** 1584, 1931.
Arch Internat. Physiol. **36:** 167, 1933.
Bayer, G. and T. Wense. Arch. exper. Path. u. Pharmakol. **188:** 114, 1937.
Arch. Internat. Pharm. Therap. **58:** 103, 1938.
Bonhomme, F. Arch. Internat. Physiol. **43:** 341, 1936.
Engelhart, E. Pflüger's Arch. **227:** 220, 1931.
Rosenblueth, A. This Journal **100:** 443, 1932.
Rosenblueth, A. and W. B. Cannon. Ibid. **99:** 398, 1931.